

(FILE 'HOME' ENTERED AT 08:55:23 ON 01 MAR 2000)

FILE 'MEDLINE, BIOSIS, SCISEARCH' ENTERED AT 08:55:32 ON 01 MAR 2000

L4 13 S METHOD AND INSERT? AND NUCLEIC AND VECTOR AND CIRCULAR?  
L5 13 DUP REM L4 (0 DUPLICATES REMOVED)  
L6 66 S VECTOR AND NUCLEIC AND MIXTURE  
L7 24 S L6 AND FRAGMENT  
L8 22 DUP REM L7 (2 DUPLICATES REMOVED)  
L9 1 S L8 AND CIRCULAR?  
L11 294 S LINEAR?()VECTOR  
L12 12 S L11 AND BLUNT  
L13 5 DUP REM L12 (7 DUPLICATES REMOVED)

=> d ibib abs 19 1

L5 ANSWER 1 OF 13 MEDLINE

ACCESSION NUMBER: 97438510 MEDLINE

DOCUMENT NUMBER: 97438510

TITLE: Construction of the temperature-sensitive \*\*\*vectors\*\*\*  
pLUC80 and pLUC88 for delivery of Tn917::NotI/SmaI and  
use of these \*\*\*vectors\*\*\* to derive a \*\*\*circular\*\*\*  
map of *Listeria monocytogenes* Scott A, a serotype 4b  
isolate.

AUTHOR: He W; Luchansky J B

CORPORATE SOURCE: Department of Food Microbiology and Toxicology, University  
of Wisconsin, Madison 53706, USA.

SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1997 Sep) 63 (9)  
3480-7.

Journal code: 6K6. ISSN: 0099-2240.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199712

AB A physical map of *Listeria monocytogenes* Scott A was generated by the  
pulsed-field technique of contour-clamped-homogeneous-electric-field  
(CHEF) electrophoresis. The \*\*\*circular\*\*\* genome of this serotype 4b  
strain contains 12 AscI fragments (38 to 790 kb), 5 NotI fragments (55 to  
1,400 kb), 3 SrfI fragments (110, 1,110, and 2,000 kb), and 2 SfiI  
fragments (1,320 and 1,920 kb). Summation of individually sized fragments  
derived by digestion of Scott A genomic DNA with each of these four  
enzymes provided an average estimated genome length of 3,210 +/- 60 kb.  
Efforts to assemble the macrorestriction map benefited greatly from the  
construction and use of pLUC80 and pLUC88, temperature-sensitive  
\*\*\*vectors\*\*\* for delivering transposon Tn917::NotI/SmaI to the  
chromosome of Scott A. As another component of this study, the positions  
of four known virulence genes (*inlA*, *mpl*, *hly*, and *prf*) and three *L.*  
*monocytogenes*-specific sequences (*lism44*, *lism51*, and *lism52*) were  
localized on the physical map of Scott A by hybridization. Probes prepared  
from *lism44*, *lism51*, and the four virulence genes hybridized within a  
cluster on a 150-kb fragment of the Scott A genome that overlaps part of  
the NotI-B and AscI-D fragments. The *lism52* probe hybridized with the  
AscI-F2 (120-kb) fragment of Scott A, which is separated from the  
NotI-B-AscI-D region by about 300 kb. These results established the first

physical and genetic map of a serotype 4b strain of *L. monocytogenes* and provided further insight on this important food-borne pathogen at the genome level.

L5 ANSWER 2 OF 13 MEDLINE

ACCESSION NUMBER: 96389016 MEDLINE  
DOCUMENT NUMBER: 96389016  
TITLE: Bacterial artificial chromosome cloning and mapping of a 630-kb human extrachromosomal structure.  
AUTHOR: Wang M; Shouse S; Lipes B; Kim U J; Shizuya H; Lai E  
CORPORATE SOURCE: Department of Pharmacology, University of North Carolina at Chapel Hill 27599-7365, USA.  
CONTRACT NUMBER: R29-GM45943 (NIGMS)  
SOURCE: GENOME RESEARCH, (1996 Jul) 6 (7) 612-9.  
Journal code: CES.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199701  
ENTRY WEEK: 19970104

AB We have cloned and mapped a \*\*\*circular\*\*\* 630-kb human extrachromosomal structure (termed amplisome) using the bacterial artificial chromosome (BAC) cloning system. Twenty-one BACs were isolated from an amplisome-enriched library by colony hybridization. The \*\*\*insert\*\*\* sizes range from 25 to 143 kb, with an average size of 82 kb. The coverage of the amplisome in clones is approximately 2.7-fold. To construct a physical map of the amplisome, we used three different but complementary \*\*\*methods\*\*\* : hybridization, STS content mapping, and fingerprinting. In addition, we compared the advantages and the drawbacks of these techniques in mapping the amplisomal BACs. The 21 BACs were grouped into two contigs and the two small gaps (3.5 and 26.5 kb) were filled by screening of a human genomic BAC library. The organization of the amplisome revealed by the BAC-based physical map is consistent with the long-range restriction map reported previously. Our results demonstrate that a 630-kb region can be rapidly cloned and mapped into contigs by use of the BAC system. Because of the low frequency (<0.1%) of chimerism and rearrangement, these BAC clones are ready for DNA sequencing and functional analysis.

L5 ANSWER 3 OF 13 MEDLINE

ACCESSION NUMBER: 96248432 MEDLINE  
DOCUMENT NUMBER: 96248432  
TITLE: Guanine-rich oligonucleotides targeted to a critical R . Y site located in the Ki-ras promoter. The effect of competing self-structures on triplex formation.  
AUTHOR: Alunni-Fabbroni M; Manzini G; Quadrifoglio F; Xodo L E  
CORPORATE SOURCE: Department of Biochemistry, Biophysics and Macromolecular Chemistry, University of Trieste, Italy.  
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (1996 May 15) 238 (1) 143-51.  
Journal code: EMZ. ISSN: 0014-2956.  
PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; Cancer Journals  
ENTRY MONTH: 199610

AB The promoter of the murine Ki-ras proto-oncogene contains a (C+G)-rich homopurine . homopyrimidine (R . Y) sequence that is essential for transcription activity. We have designed two G-rich oligonucleotides, d(TGGGTGGGTGGTTGGGTGGG) (20GT) and d(AGGGAGGGAGGAAGGGAGGG) (20AG), that have the potential to bind the critical Ki-ras sequence via triplex-helix formation. Band-shift experiments have shown that 20GT binds the Ki-ras R . Y duplex with a delta G value of -40 +/- 5 kJ/mol, while 20AG appeared to have a lower affinity under the experimental conditions adopted: 50 mM Tris/HCl, pH 7.4, 50 mM NaCl, 5 mM MgCl<sub>2</sub>, 25 degrees C. In the absence of Mg<sup>2+</sup>, 20GT did not bind to the Ki-ras R . Y target, while 20AG exhibited the same affinity observed in the magnesium-containing buffer. To gain insight into the solution properties of 20GT and 20AG, we have performed several experiments including polyacrylamide gel electrophoresis (PAGE), hydroxyapatite chromatography, ultraviolet absorption melting and \*\*\*circular\*\*\* dichroism (CD). We found that 20AG rapidly self-associates into presumably a duplex, even at low concentration (< 1 microM), while 20GT forms aggregates slowly, a process favoured by high oligonucleotide concentrations (> 25 microM). The critical Ki-ras sequence was \*\*\*inserted\*\*\* in Bluescript KS+, downstream from the T7 promoter, to investigate to what extent 20AG and 20GT, which are directed against the R . Y target, are able to inhibit T7 RNA polymerase transcription, under near-physiological conditions. Transcription experiments conducted in vitro at pH 7.4 have shown that oligonucleotide 20GT produced a remarkable repression of T7 RNA polymerase activity in the concentration range (10-25 microM), whereas 20AG had little effect on transcription. In conclusion, the results of this work together with other data reported in the literature [Olivas, W. M. & Maher, L. J. III (1995) Biochemistry 34, 278-284; Noonberg, S. B., Francois, J.-C., Garestier, T. & Hel'ene, C. (1995) \*\*\*Nucleic\*\*\* Acids Res. 23, 1956-1963], demonstrate that G-rich oligonucleotides, in particular (G,A)-sequences, may raise problems for in vivo application due to self-aggregation.

L5 ANSWER 4 OF 13 MEDLINE

ACCESSION NUMBER: 96200853 MEDLINE

DOCUMENT NUMBER: 96200853

TITLE: In vivo intermolecular recombination in Escherichia coli: application to plasmid constructions.

AUTHOR: Degryse E

CORPORATE SOURCE: Yeast Department, Transg'ene SA, Strasbourg, France.

SOURCE: GENE, (1996 Apr 17) 170 (1) 45-50.

Journal code: FOP. ISSN: 0378-1119.

PUB. COUNTRY: Netherlands

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199608

AB Repair of a double-strand break (DSB) was investigated by intermolecular recombination in Escherichia coli (Ec) recBC sbcBC cells with restriction enzyme-cleaved model plasmids. \*\*\*Circular\*\*\* plasmids were generated when a linearized plasmid ( \*\*\*vector\*\*\* ) containing an origin of replication was co-transformed with a DNA fragment (template) containing a homologous sequence. The influence of the position of the DSB in the \*\*\*vector\*\*\* was analyzed using templates which contain various genetic markers, non-homologous sequences and/or deletions relative to the \*\*\*vector\*\*\*. In all cases, when a DSB occurs within a marker, this marker is lost in the resulting plasmid, whereas markers flanked by homologous regions located in the vicinity of a DSB are transmitted.

\*\*\*Insertions\*\*\* (deletions), substitutions and shuffling of genetic markers are possible by in vivo recombination using Ec and can be applied to plasmid constructions. It is shown that recombination can occur from both template ends or from one \*\*\*vector\*\*\* and one template end. A D-loop nuclease is suggested to participate in the resolution of the recombination intermediates.

L5 ANSWER 5 OF 13 MEDLINE

ACCESSION NUMBER: 96036200 MEDLINE  
DOCUMENT NUMBER: 96036200  
TITLE: Study of the organization of the genomes of Escherichia coli, Brucella melitensis and Agrobacterium tumefaciens by \*\*\*insertion\*\*\* of a unique restriction site.  
AUTHOR: Jumas-Bilak E; Maugard C; Michaux-Charachon S; Allardet-Servent A; Perrin A; O'Callaghan D; Ramuz M  
CORPORATE SOURCE: Institut National de la Sante et de la Recherche Medicale, Unite 431, Faculte de Medecine, Nimes, France..  
SOURCE: MICROBIOLOGY, (1995 Oct) 141 ( Pt 10) 2425-32.  
Journal code: .BXW. ISSN: 1350-0872.  
PUB. COUNTRY: ENGLAND: United Kingdom  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199602

AB Tn5Map, a Tn5 derivative containing the 18 bp I-SceI site, was delivered from a RP4-mobilizable, RK6-derived suicide \*\*\*vector\*\*\* to Escherichia coli HB101, Brucella melitensis and Agrobacterium tumefaciens C58, which all lack natural I-SceI sites in their genomes. Digestion of the DNA from Tn5Map-containing strains and analysis by pulsed-field gel electrophoresis (PFGE) revealed that these derivatives contained a single transposon \*\*\*insertion\*\*\*. These digests also gave direct and independent proof for the single \*\*\*circular\*\*\* chromosome of E. coli, and for the presence of two \*\*\*circular\*\*\* chromosomes in B. melitensis and of a \*\*\*circular\*\*\* and a linear chromosome in A. tumefaciens C58 (which also contains two large \*\*\*circular\*\*\* plasmids). This rapid and versatile technique is potentially applicable to the study of the genomic organization in all Gram-negative bacteria which support Tn5 transposition. Moreover, linearization of \*\*\*circular\*\*\* replicons could be the first step for a rapid \*\*\*method\*\*\* of physical mapping.

L5 ANSWER 6 OF 13 MEDLINE

ACCESSION NUMBER: 96000006 MEDLINE  
DOCUMENT NUMBER: 96000006  
TITLE: Minimal length requirement of the single-stranded tails for ligation-independent cloning (LIC) of PCR products.  
AUTHOR: Aslanidis C; de Jong P J; Schmitz G  
CORPORATE SOURCE: Institute for Clinical Chemistry, University of Regensburg, Germany.  
SOURCE: PCR METHODS AND APPLICATIONS, (1994 Dec) 4 (3) 172-7.  
Journal code: BNV. ISSN: 1054-9803.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199602

AB The ligation-independent cloning of PCR products (LIC-PCR) is a versatile

and highly efficient cloning procedure resulting in recombinant clones only. Recombinants are generated between PCR products and a PCR-amplified \*\*\*vector\*\*\* through defined complementary single-stranded (ss) ends artificially generated with T4 DNA polymerase. This procedure does not require restriction enzymes, alkaline phosphatase, or DNA ligase. The primers used for amplification contain an additional 12-nucleotide sequence at their 5' ends that is complementary in the \*\*\*vector\*\*\* - and \*\*\*insert\*\*\* -specific primers. The (3'-->5') exonuclease activity of T4 DNA polymerase is used in combination with a predetermined dNTP (dGTP for the \*\*\*inserts\*\*\* and dCTP for the \*\*\*vector\*\*\* ) to specifically remove 12 nucleotides from each 3' end of the PCR fragments. Because of the complementarity of the ends that are generated, \*\*\*circularization\*\*\* can occur between \*\*\*vector\*\*\* and \*\*\*insert\*\*\* . The recombinant molecules do not require in vitro ligation for efficient bacterial transformation. To make this technique widely applicable, we have simplified the handling of the PCR fragments prior to LIC. The PCR products do not need further purification following the T4 DNA polymerase treatment. Incubation of \*\*\*vector\*\*\* and \*\*\*insert\*\*\* PCR fragments for as little as 5 min is sufficient for a high yield of recombinants. Comparison of the transformation efficiencies using different-length LIC tails revealed that using 12-nucleotide cohesive ends produced four times more transformants than were obtained with the LIC with 10-nucleotide cohesive ends. When the LIC tails were 8 nucleotides long, no transformants were obtained. PCR fragment purification, T4 DNA polymerase treatment, and LIC is complete in < 1 hr.

L5 ANSWER 7 OF 13 MEDLINE

ACCESSION NUMBER: 92228797 MEDLINE

DOCUMENT NUMBER: 92228797

TITLE: Construction of small- \*\*\*insert\*\*\* genomic DNA libraries highly enriched for microsatellite repeat sequences.

AUTHOR: Ostrand E A; Jong P M; Rine J; Duyk G

CORPORATE SOURCE: Department of Molecular and Cellular Biology, University of California, Berkeley 94720.

CONTRACT NUMBER: ESO1896

SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1992 Apr 15) 89 (8) 3419-23. Journal code: PV3. ISSN: 0027-8424.

PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; Cancer Journals

ENTRY MONTH: 199207

AB We describe an efficient \*\*\*method\*\*\* for the construction of small- \*\*\*insert\*\*\* genomic libraries enriched for highly polymorphic, simple sequence repeats. With this approach, libraries in which 40-50% of the members contain (CA)<sub>n</sub> repeats are produced, representing an approximately 50-fold enrichment over conventional small- \*\*\*insert\*\*\* genomic DNA libraries. Briefly, a genomic library with an average \*\*\*insert\*\*\* size of less than 500 base pairs was constructed in a phagemid \*\*\*vector\*\*\* . Amplification of this library in a dut ung strain of Escherichia coli allowed the recovery of the library as closed \*\*\*circular\*\*\* single-stranded DNA with uracil frequently incorporated in place of thymine. This DNA was used as a template for second-strand DNA synthesis, primed with (CA)<sub>n</sub> or (TG)<sub>n</sub> oligonucleotides, at elevated

temperatures by a thermostable DNA polymerase. Transformation of this mixture into wild-type E. coli strains resulted in the recovery of primer-extended products as a consequence of the strong genetic selection against single-stranded uracil-containing DNA molecules. In this manner, a library highly enriched for the targeted microsatellite-containing clones was recovered. This approach is widely applicable and can be used to generate marker-selected libraries bearing any simple sequence repeat from cDNAs, whole genomes, single chromosomes, or more restricted chromosomal regions of interest.

L5 ANSWER 8 OF 13 MEDLINE

ACCESSION NUMBER: 93142966 MEDLINE

DOCUMENT NUMBER: 93142966

TITLE: Cycled DNA immunoprecipitation procedure to enrich the target sequences for DNA binding proteins with the fold purification monitored.

AUTHOR: Sugimoto K; Wakisaka E; Himeno M

CORPORATE SOURCE: Department of Agricultural Chemistry, College of Agriculture, University of Osaka Prefecture, Japan..

SOURCE: ANALYTICAL BIOCHEMISTRY, (1992 Nov 15) 207 (1) 114-20.  
Journal code: 4NK. ISSN: 0003-2697.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199304

AB Using centromere DNA binding protein (CENP-B) expressed as a fusion to beta-galactosidase in Escherichia coli, we established a cycled DNA immunoprecipitation procedure for enriching CENP-B binding sequences and monitoring the enrichment process. Degenerated synthetic oligonucleotides for an authentic CENP-B binding sequence, \*\*\*inserted\*\*\* into a pUC-derived \*\*\*vector\*\*\*, were incubated with the crude CENP-B extract. DNA-protein complexes formed in vitro were immunologically precipitated utilizing the beta-galactosidase moiety as a tagged antigen. The effectiveness of repeating cycles of immunoprecipitation was demonstrated by the color selection \*\*\*method\*\*\* designed for pUC-derived plasmids, after introducing the precipitated plasmids into Escherichia coli. After three cycles of DNA immunoprecipitation, only a few kinds of sequences constituted the majority. By repeating two more cycles, the most predominant sequence was finally enriched until homogeneous, indicating the enrichment of the binding sequences in a hierarchical order. Further application to human genomic DNA showed that two EcoRI DNA fragments, 0.49 and 0.78 kb in size, were exclusively identified. This procedure can be applied to the systematic analysis of binding sequences for any other DNA binding proteins without production of any specific antibodies or further purification.

L5 ANSWER 9 OF 13 MEDLINE

ACCESSION NUMBER: 92008483 MEDLINE

DOCUMENT NUMBER: 92008483

TITLE: Detection and identification of Leishmania parasites by in situ hybridization with total and recombinant DNA probes.

AUTHOR: Schoone G J; van Eys G J; Ligthart G S; Taub F E; Zaal J; Mebrahtu Y; Laywer P

CORPORATE SOURCE: Laboratory of Tropical Hygiene, Royal Tropical Institute, Amsterdam, The Netherlands..

SOURCE: EXPERIMENTAL PARASITOLOGY, (1991 Oct) 73 (3) 345-53.

Journal code: EQP. ISSN: 0014-4894.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199201

AB In situ hybridization on cultured promastigotes and sandfly smears were performed with nonradioactively labeled total DNA and recombinant DNA probes containing minicircle kinetoplast DNA (kDNA) or nuclear DNA \*\*\*inserts\*\*\*. Total DNA probes lack specificity whereas recombinant nuclear DNA probes work only if they contain repetitive sequences. Minicircle kDNAs of five Leishmania isolates, representative of five Leishmania taxa found in Kenya, were sequenced. Comparison of the sequences showed a 150-bp region with around 80% homology, whereas the rest of the minicircles had about 50% homology. Nevertheless, application of these probes in in situ hybridization assays as tested on Leishmania promastigotes in the \*\*\*vector\*\*\* gave good specificity and hybridization signal. Two types of labeling were tested: incorporation of biotin-labeled dUTP or directly horseradish peroxidase (HRP)-labeled nucleotides. Both techniques provided good sensitivity and signal-to-noise ratio on cultured promastigotes. Hybridization with HRP-labeled kDNA probes gave a superior signal-to-noise ratio if tested on sandfly preparations. This \*\*\*method\*\*\* provided a reliable and fast identification and facilitated the detection of promastigotes in sandflies. The technique presented here may be helpful in rapid identification of Leishmania promastigotes, and thus make epidemiological studies easier and less time consuming.

L5 ANSWER 10 OF 13 MEDLINE

ACCESSION NUMBER: 91081323 MEDLINE  
DOCUMENT NUMBER: 91081323  
TITLE: Site-directed, recombination-mediated mutagenesis of a complex gene locus.  
AUTHOR: Barton M C; Hoekstra M F; Emerson B M  
CORPORATE SOURCE: Regulatory Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037.  
CONTRACT NUMBER: GM38760 (NIGMS)  
SOURCE: NUCLEIC ACIDS RESEARCH, (1990 Dec 25) 18 (24) 7349-55.  
Journal code: O8L. ISSN: 0305-1048.  
PUB. COUNTRY: ENGLAND: United Kingdom  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; Cancer Journals  
ENTRY MONTH: 199104

AB We have generated a site-specific 17 bp \*\*\*insertion\*\*\* within a 38 kb chick globin gene cluster by employing the recombination abilities of Saccharomyces cerevisiae. This gene cluster contains four beta-type globin genes which share a high degree of sequence homology. In this procedure, a small fragment of beta A-globin DNA containing a 17 bp \*\*\*insertion\*\*\* is subcloned into a URA3-based yeast integrating \*\*\*vector\*\*\* (YIp). This mutated globin subclone is introduced into cells that carry the 38 kb globin cluster clone on a single-copy, \*\*\*circular\*\*\* \*\*\*vector\*\*\* derived from a yeast artificial chromosome (YAC). \*\*\*Insertion\*\*\* of the 17 bp oligomer is achieved by targeted integration of the YIp subclone. The recombinant contains the normal beta A-globin gene, the mutant gene and YIp \*\*\*vector\*\*\* sequences between the two copies. Excision of the \*\*\*vector\*\*\* sequences and one copy of the duplicated

globin sequences by homologous recombination is required for cell survival when exposed to the selective agent 5-fluoroorotic acid, which is toxic to ura<sup>+</sup> yeast cells. Depending upon the point of the cross-over, a ura<sup>-</sup> yeast cell bearing either a wild-type globin gene or a 17 bp \*\*\*insertion\*\*\* mutation will result. By restriction mapping and in vitro transcription analysis, the beta A-globin gene containing the 17 bp \*\*\*insert\*\*\* has no nonspecific mutations generated during the recombination and selection procedures. Specific mutations of regulatory regions, including protein-DNA binding sites, can be accurately targeted within extensive DNA clones by this \*\*\*method\*\*\* .

L5 ANSWER 11 OF 13 MEDLINE

ACCESSION NUMBER: 90076959 MEDLINE

DOCUMENT NUMBER: 90076959

TITLE: An efficient directional cloning system to construct cDNA libraries containing full-length \*\*\*inserts\*\*\* at high frequency.

AUTHOR: Miki T; Matsui T; Heidaran M A; Aaronson S A

CORPORATE SOURCE: Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD 20892..

SOURCE: GENE, (1989 Nov 15) 83 (1) 137-46.

Journal code: FOP. ISSN: 0378-1119.

PUB. COUNTRY: Netherlands

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-M30493; GENBANK-M30494

ENTRY MONTH: 199003

AB We have developed a high efficiency cDNA cloning system which can direct the orientation of \*\*\*inserts\*\*\* in lambda-plasmid composite \*\*\*vectors\*\*\* with large cloning capacities. Cleavage of the \*\*\*vector\*\*\* DNA by SfiI creates two different nonsymmetrical 3' extensions at the ends of the \*\*\*vector\*\*\* arms. Using a linker-primer and an adaptor, cDNA is prepared so it has two different sticky ends which can be ligated to those of the \*\*\*vector\*\*\* arms. When the cDNA fragments and the \*\*\*vector\*\*\* arms are mixed, both the molecules can assemble without self- \*\*\*circularization\*\*\* due to base-pairing specificity. Ligation of the cDNA- \*\*\*vector\*\*\* mixture produces the concatemers from which phage clones carrying a single cDNA \*\*\*insert\*\*\* in the desired orientation can be formed very efficiently by in vitro packaging. This system provides: (1) high cloning efficiency [10(7)-10(8) clones/micrograms poly(A)+ RNA], (2) low background (more than 90% of the clones contain \*\*\*inserts\*\*\*), (3) directional \*\*\*insertion\*\*\* of cDNA fragments into the \*\*\*vectors\*\*\*, (4) presence of a single \*\*\*insert\*\*\* in each clone, (5) accommodation of long \*\*\*inserts\*\*\* (up to 10 kb), (6) a mechanism for rescue of the plasmid part from the lambda genome, and (7) a straightforward protocol for library preparation. Screenings of cDNA libraries constructed by this \*\*\*method\*\*\* demonstrated that cDNAs of up to 6.4 kb, containing complete coding sequences, could be isolated at high efficiency. Thus, this cloning system should be useful for the isolation of cDNAs of relatively long transcripts, present even at low abundance, in cells.

L5 ANSWER 12 OF 13 MEDLINE

ACCESSION NUMBER: 85297751 MEDLINE

DOCUMENT NUMBER: 85297751

TITLE: Separation of complementary strands of plasmid DNA using

the biotin-avidin system and its application to heteroduplex formation and RNA/DNA hybridizations in electron microscopy.

AUTHOR: Delius H; van Heerikhuizen H; Clarke J; Koller B  
SOURCE: NUCLEIC ACIDS RESEARCH, (1985 Aug 12) 13 (15) 5457-69.  
Journal code: O8L. ISSN: 0305-1048.  
PUB. COUNTRY: ENGLAND: United Kingdom  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; Cancer Journals  
ENTRY MONTH: 198512

AB A \*\*\*method\*\*\* for the separation of complementary strands with the help of the biotin-avidin system is described. Restriction fragments were terminally labeled at both ends with biotinylated nucleotides. The DNA was cut by a second restriction enzyme, and the fragments were bound to an avidin agarose column. The non-biotinylated strands were eluted with 0.1 M NaOH, and the biotin-labeled strands were subsequently released from the column by elution with 50% guanidine isothiocyanate/formamide. Contamination of the separated strands by complementary single strands was less than 4%.--Separated linear single strands of the \*\*\*vector\*\*\* pEMBL were prepared. On annealing with recombinant \*\*\*circular\*\*\* DNA a substitution loop is formed which provides position and orientation markers for the unambiguous electron microscopic analysis of heteroduplexes or hybrids formed with the \*\*\*inserted\*\*\* sequences. -The terminal biotin label was visualized by complex formation with a streptavidin-ferritin conjugate.

L5 ANSWER 13 OF 13 MEDLINE

ACCESSION NUMBER: 85076178 MEDLINE  
DOCUMENT NUMBER: 85076178  
TITLE: Oligonucleotide-directed mutagenesis: a simple  
\*\*\*method\*\*\* using two oligonucleotide primers and a single-stranded DNA template.  
AUTHOR: Zoller M J; Smith M  
SOURCE: DNA, (1984 Dec) 3 (6) 479-88.  
Journal code: EAW. ISSN: 0198-0238.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198504

AB This paper presents a simple and efficient \*\*\*method\*\*\* for oligonucleotide-directed mutagenesis using \*\*\*vectors\*\*\* derived from single-stranded phage. This modification of our previously published procedure (Zoller and Smith, 1982) features the use of two primers, one of which is a standard M13 sequencing primer and the other is the mutagenic oligonucleotide. Both primers are simultaneously annealed to single-stranded template DNA, extended by DNA polymerase I (large fragment), and ligated together to form a mutant wild-type gapped heteroduplex. Escherichia coli is transformed directly with this DNA; the isolation of covalently closed \*\*\*circular\*\*\* DNA as in our previous report is not necessary. Mutants are identified by plaque lift hybridization using the mutagenic oligonucleotide as a probe. As an example of the \*\*\*method\*\*\*, a heptadecanucleotide was used to create a T----G transversion in the MATA gene of Saccharomyces cerevisiae cloned into the \*\*\*vector\*\*\* M13mp5. The efficiency of mutagenesis was approximately 50%. Production of the desired mutation was verified by DNA

sequencing. The same procedure has been used without modification to create \*\*\*insertions\*\*\* of restriction sites as well as specific deletions of 500 bases.

L9 ANSWER 1 OF 1 MEDLINE  
ACCESSION NUMBER: 90076959 MEDLINE  
DOCUMENT NUMBER: 90076959  
TITLE: An efficient directional cloning system to construct cDNA libraries containing full-length inserts at high frequency.  
AUTHOR: Miki T; Matsui T; Heidaran M A; Aaronson S A  
CORPORATE SOURCE: Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD 20892..  
SOURCE: GENE, (1989 Nov 15) 83 (1) 137-46.  
Journal code: FOP. ISSN: 0378-1119.  
PUB. COUNTRY: Netherlands  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-M30493; GENBANK-M30494  
ENTRY MONTH: 199003

AB We have developed a high efficiency cDNA cloning system which can direct the orientation of inserts in lambda-plasmid composite \*\*\*vectors\*\*\* with large cloning capacities. Cleavage of the \*\*\*vector\*\*\* DNA by SfiI creates two different nonsymmetrical 3' extensions at the ends of the \*\*\*vector\*\*\* arms. Using a linker-primer and an adaptor, cDNA is prepared so it has two different sticky ends which can be ligated to those of the \*\*\*vector\*\*\* arms. When the cDNA \*\*\*fragments\*\*\* and the \*\*\*vector\*\*\* arms are mixed, both the molecules can assemble without self-\*\*\*circularization\*\*\* due to base-pairing specificity. Ligation of the cDNA-\*\*\*vector\*\*\* \*\*\*mixture\*\*\* produces the concatemers from which phage clones carrying a single cDNA insert in the desired orientation can be formed very efficiently by in vitro packaging. This system provides: (1) high cloning efficiency [10<sup>(7)</sup>-10<sup>(8)</sup> clones/micrograms poly(A)+ RNA], (2) low background (more than 90% of the clones contain inserts), (3) directional insertion of cDNA \*\*\*fragments\*\*\* into the \*\*\*vectors\*\*\*, (4) presence of a single insert in each clone, (5) accommodation of long inserts (up to 10 kb), (6) a mechanism for rescue of the plasmid part from the lambda genome, and (7) a straightforward protocol for library preparation. Screenings of cDNA libraries constructed by this method demonstrated that cDNAs of up to 6.4 kb, containing complete coding sequences, could be isolated at high efficiency. Thus, this cloning system should be useful for the isolation of cDNAs of relatively long transcripts, present even at low abundance, in cells.

=> d ibib abs 113 1-4

L13 ANSWER 1 OF 5 MEDLINE DUPLICATE 1  
ACCESSION NUMBER: 97449307 MEDLINE  
DOCUMENT NUMBER: 97449307  
TITLE: Cloning of linear DNAs in vivo by overexpressed T4 DNA ligase: construction of a T4 phage hoc gene display vector.  
AUTHOR: Ren Z J; Baumann R G; Black L W  
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology,

University of Maryland School of Medicine, Baltimore  
21201-1503, USA.

CONTRACT NUMBER: AI-11676 (NIAID)  
SOURCE: GENE, (1997 Aug 22) 195 (2) 303-11.  
Journal code: FOP. ISSN: 0378-1119.  
PUB. COUNTRY: Netherlands  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199801  
ENTRY WEEK: 19980104

AB A method was developed to clone linear DNAs by overexpressing T4 phage DNA ligase in vivo, based upon recombination deficient E. coli derivatives that carry a plasmid containing an inducible T4 DNA ligase gene. Integration of this ligase-plasmid into the chromosome of such E. coli allows standard plasmid isolation following linear DNA transformation of the strains containing high levels of T4 DNA ligase. Intramolecular ligation allows high efficiency recircularization of cohesive and \*\*\*blunt\*\*\* -end terminated linear plasmid DNAs following transformation.

Recombinant plasmids could be constructed in vivo by co-transformation with \*\*\*linearized\*\*\* \*\*\*vector\*\*\* plus insert DNAs, followed by intermolecular ligation in the T4 ligase strains to yield clones without deletions or rearrangements. Thus, in vitro packaged lox-site terminated plasmid DNAs injected from phage T4 were recircularized by T4 ligase in vivo with an efficiency comparable to CRE recombinase. Clones that expressed a capsid-binding 14-aa N-terminal peptide extension derivative of the HOC (highly antigenic outer capsid) protein for T4 phage hoc gene display were constructed by co-transformation with a \*\*\*linearized\*\*\* \*\*\*vector\*\*\* and a PCR-synthesized hoc gene. Therefore, the T4 DNA ligase strains are useful for cloning linear DNAs in vivo by transformation or transduction of DNAs with nonsequence-specific but compatible DNA ends.

L13 ANSWER 2 OF 5 MEDLINE DUPLICATE 2  
ACCESSION NUMBER: 97135101 MEDLINE  
DOCUMENT NUMBER: 97135101  
TITLE: Construction of a vector containing a site-specific DNA double-strand break with 3'-phosphoglycolate termini and analysis of the products of end-joining in CV-1 cells.  
AUTHOR: Bennett R A; Gu X Y; Povirk L F  
CORPORATE SOURCE: Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond 23298, USA.  
CONTRACT NUMBER: CA40615 (NCI)  
SOURCE: INTERNATIONAL JOURNAL OF RADIATION BIOLOGY, (1996 Dec) 70 (6) 623-36.  
Journal code: IRB. ISSN: 0955-3002.  
PUB. COUNTRY: ENGLAND: United Kingdom  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; Cancer Journals  
ENTRY MONTH: 199703  
ENTRY WEEK: 19970304

AB Previous studies have shown that linearized SV40-based shuttle vectors transfected into mammalian cells are efficiently recircularized by an error-prone end-joining pathway. To determine whether and with what

specificity free radical-mediated double-strand breaks are rejoined by this pathway, a structural mimic of such a break was introduced at a specific site in an SV40-based shuttle vector, by ligating purified 3'-phosphoglycolate-terminated oligonucleotides into 3' recessed ends generated in the \*\*\*linearized\*\*\* \*\*\*vector\*\*\*. These terminally blocked \*\*\*linear\*\*\* \*\*\*vectors\*\*\* were efficiently repaired and replicated when transfected into simian CV-1 cells. Sequencing across the repair joints in progeny plasmid indicated that, for a \*\*\*blunt\*\*\* -ended vector, the most frequent mechanism of rejoining was splicing at a terminal 4-base homology; however, a significant fraction of the joints retained all bases from both ends of the break, consistent with a mechanism involving simple 3'-phosphoglycolate removal, followed by \*\*\*blunt\*\*\* -end ligation. For the analogous 3'-hydroxyl terminated break, the fraction of simple \*\*\*blunt\*\*\* -end ligations was considerably higher. For a phosphoglycolate-terminated vector with cohesive ends the most frequent repair mechanism was simple ligation of the annealed cohesive ends, presumably preceded by phosphoglycolate removal. For all these substrates, the remaining repair joints showed small or large deletions from one or both of the ends, usually with apparent annealing at short (1-4-base) homologies. The results suggest that while breaks with 3'-phosphoglycolates can be repaired, these blocked termini represent a significant barrier to DNA end-joining, and can significantly alter its specificity. The presence of cohesive ends appears to improve markedly the fidelity of rejoining for terminally blocked double-strand breaks.

L13 ANSWER 3 OF 5 MEDLINE DUPLICATE 3  
 ACCESSION NUMBER: 94031045 MEDLINE  
 DOCUMENT NUMBER: 94031045  
 TITLE: Directional cloning of \*\*\*blunt\*\*\* -ended PCR products.  
 AUTHOR: Weiner M P  
 CORPORATE SOURCE: Stratagene Cloning Systems, La Jolla, CA 92037.  
 SOURCE: BIOTECHNIQUES, (1993 Sep) 15 (3) 502-5.  
 Journal code: AN3. ISSN: 0736-6205.  
 PUB. COUNTRY: United States  
 Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199402

AB A method that allows the directional cloning of \*\*\*blunt\*\*\* -ended polymerase chain reaction (PCR) fragments is described. One PCR primer must be 5' phosphorylated. Extra bases are not required on either PCR primer. A \*\*\*linearized\*\*\* \*\*\*vector\*\*\* is enzymatically processed to contain a single 5'-terminal phosphate. The monophosphorylated vector is amenable to recombinant-insertion during ligation when the fragment is in the correct orientation. Increased recombinant yield results from incubating the monophosphorylated vector with a restriction enzyme (SrfI) that relinearizes nonrecombinant plasmids during the ligation reaction.

L13 ANSWER 4 OF 5 MEDLINE DUPLICATE 4  
 ACCESSION NUMBER: 94031037 MEDLINE  
 DOCUMENT NUMBER: 94031037  
 TITLE: A simple two-step method for efficient \*\*\*blunt\*\*\* -end ligation of DNA fragments.  
 AUTHOR: Damak S; Bullock D W  
 CORPORATE SOURCE: Centre for Molecular Biology, Lincoln University, Canterbury, New Zealand.

SOURCE: BIOTECHNIQUES, (1993 Sep) 15 (3) 448-50, 452.  
Journal code: AN3. ISSN: 0736-6205.  
PUB. COUNTRY: United States  
Report; (TECHNICAL REPORT)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199402

AB The formation of recombinant plasmids results from ligation between one end of the \*\*\*linearized\*\*\* \*\*\*vector\*\*\* and one end of the insert (favored by high DNA concentration), followed by self-ligation of the newly created hybrid molecule (favored by low DNA concentration). Standard protocols recommend an average DNA concentration at which both events may occur. Since this DNA concentration is not optimum for both ligation events, efficient \*\*\*blunt\*\*\* -end ligation is compromised. We describe a method for \*\*\*blunt\*\*\* -end ligation starting at a high DNA concentration for 1 h then at 1/20 the initial DNA concentration overnight. The number of recombinant plasmids obtained with this method is about 10-fold higher than with standard protocols. Restriction digestion and agarose gel electrophoresis of 10 recombinant plasmids obtained with the two-step ligation method showed that all plasmids contained one copy of the insert.

=> d his

(FILE 'HOME' ENTERED AT 12:34:17 ON 01 MAR 2000)

FILE 'BIOSIS, SCISEARCH, MEDLINE, CAPLUS' ENTERED AT 12:34:27 ON 01 MAR 2000

L1 93 S CIRCULAR?()VECTOR?  
L2 42 DUP REM L1 (51 DUPLICATES REMOVED)

=> d ibib abs 12 1-42

L2 ANSWER 1 OF 42 SCISEARCH COPYRIGHT 2000 ISI (R) DUPLICATE 1  
ACCESSION NUMBER: 2000:106849 SCISEARCH  
THE GENUINE ARTICLE: 280KH  
TITLE: Circular YAC vectors containing short mammalian origin sequences are maintained under selection as HeLa episomes  
AUTHOR: Nielsen T O; Cossons N H; ZannisHadjopoulos M; Price G B (Reprint)  
CORPORATE SOURCE: MCGILL UNIV, MCGILL CANC CTR, 3655 DRUMMOND ST, MONTREAL, PQ H3G 1Y6, CANADA (Reprint); MCGILL UNIV, MCGILL CANC CTR, MONTREAL, PQ H3G 1Y6, CANADA; UNIV BRITISH COLUMBIA, DEPT PATHOL, VANCOUVER, BC V6T 1Z3, CANADA  
COUNTRY OF AUTHOR: CANADA  
SOURCE: JOURNAL OF CELLULAR BIOCHEMISTRY, (JAN 2000) Vol. 76, No. 4, pp. 674-685.  
Publisher: WILEY-LISS, DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012.  
ISSN: 0730-2312.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English

REFERENCE COUNT: 35

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB pYACneo, a 15.8-kb plasmid, contains a bacterial origin, G418-resistance gene, and yeast ARS, CEN, and TEL elements. Three mammalian origins have been cloned into this \*\*\*circular\*\*\*  
\*\*\*vector\*\*\* : 343, a 448-bp chromosomal origin from a transcribed region

of human chromosome 6q; X24, a 4.3-kb element containing the hamster DHFR origin of bidirectional replication (ori beta), and S3, a 1.1-kb human anti-cruciform purified autonomously replicating sequence. The resulting constructs have been transfected into HeLa cells, and G418-resistant subcultures were isolated. The frequency of G418-resistant transformation was 1.7-8.7 times higher with origin-containing YACneo than with vector alone. After >45 generations under G418 selection, the presence of episomal versus integrated constructs was assessed by fluctuation assay and by PCR of supercoiled, circular, and linear genomic cellular DNAs separated on ethidium bromide-caesium chloride gradients. In stable G418-resistant subcultures transfected with vector alone or with linearized constructs, as well as in some subcultures transfected with circular origin-containing constructs, resistance was conferred by integration into the host genome. However, several examples were found of G418-resistant transfectants maintaining the Y.343 and the YAC.S3 circular constructs in a strictly episomal state after long-term culture in selective medium, with 80-90% stability per cell division. The episomes were found to replicate semiconservatively in a bromodeoxyuridine pulse-labeling assay for less than or equal to 130 cell generations after transfection. Furthermore, after less than or equal to 172 cell generations rescued episomal DNA could be isolated intact and unrearranged, and could be used to retransform bacteria. These versatile constructs, containing mammalian origins, have the capacity for further modification with human telomere or large putative centromere elements, in an effort to move towards construction of a human artificial chromosome.  
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L2 ANSWER 2 OF 42 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1999:350769 CAPLUS  
DOCUMENT NUMBER: 131:1433  
TITLE: Methods and means for mutagenesis of DNA  
INVENTOR(S): Joly, Etienne Lucien Daniel  
PATENT ASSIGNEE(S): The Babraham Institute, UK  
SOURCE: PCT Int. Appl., 56 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9925871	A1	19990527	WO 1998-GB3461	19981117
W: JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				

PRIORITY APPLN. INFO.: GB 1997-24270 19971117

AB Methods and means for mutagenesis of DNA, particularly site-directed mutagenesis or replacement of DNA segments, are disclosed. Nucleic acid amplification, such as PCR, is used in prodn. of copies of template DNA

including one or more mutations. A parental DNA mol. includes template DNA provided within a \*\*\*circular\*\*\* \*\*\*vector\*\*\*. A pair of initial primers is used to generate further primers which are then used to copy the entire parental DNA mol. including template and vector into a form including the desired mutation or mutations. Where only one mutation is to be introduced, only one of the initial primers need be mutagenic. Two distal mutations may be introduced at the same time using two mutagenic primers. The parental DNA including original template is removed by digestion. Parental DNA may be methylated to allow for its digestion with a suitable enzyme, such as DpnI, which does not digest newly synthesized (unmethylated) product DNA. Means for performing the methods are amenable to provision in kit form.

L2 ANSWER 3 OF 42 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1999:576696 CAPLUS  
DOCUMENT NUMBER: 131:166230  
TITLE: Method for marker-free repetitive DNA expression cassette exchange in the genome of cells or parts of cells  
INVENTOR(S): Bode, Jurgen; Seibler, Jost; Schubeler, Dirk  
PATENT ASSIGNEE(S): Gesellschaft fur Biotechnologische Forschung m.b.H. (GBF), Germany  
SOURCE: Eur. Pat. Appl., 13 pp.  
CODEN: EPXXDW  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 939120	A1	19990901	EP 1998-103490	19980227
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2000023679	A2	20000125	JP 1999-50995	19990226
PRIORITY APPLN. INFO.:			EP 1998-103490	19980227

AB The invention relates to a method for marker-free repetitive DNA expression cassette exchange in the genome of cells or parts of cells by using the FLP recombinase-mediated cassette exchange (RMCE). In a first step a first DNA expression cassette carrying a pos.-neg. selection marker flanked by a wild type FLP recombinase recognition target (FRT) site on one end and a modified heterospecific FRT on the other end is integrated into a chromosomal locus of the genome for tagging. Following selection of cell clones surviving the conditions for pos. selection said first DNA cassette as a second step is exchanged by an incoming second DNA expression cassette located on a \*\*\*circular\*\*\* \*\*\*vector\*\*\* and carrying a homologous or heterologous gene (transgene) of any coding sequence flanked by the same FRT sites as the first DNA cassette by using FLP-recombinase. The cell clones surviving the conditions for neg. selection contain specifically inserted the gene of the incoming DNA cassette without inserted unwanted vector sequences or pos. selectable markers.

L2 ANSWER 4 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS

DUPLICATE 2

ACCESSION NUMBER: 1999:192092 BIOSIS  
DOCUMENT NUMBER: PREV199900192092  
TITLE: Latrunculin-A causes mydriasis and cycloplegia in the

cynomolgus monkey.

AUTHOR(S): Peterson, Jennifer A.; Tian, Baohe; Geiger, Benjamin; Kaufman, Paul L. (1)

CORPORATE SOURCE: (1) Department of Ophthalmology and Visual Sciences, 600 Highland Avenue, Madison, WI, 53792-3220 USA

SOURCE: IOVS, (March, 1999) Vol. 40, No. 3, pp. 631-638.

DOCUMENT TYPE: Article

LANGUAGE: English

AB Purpose. To determine the effect of latrunculin (LAT)-A, which binds to G-actin and disassembles actin filaments, on the pupil, accommodation, and isolated ciliary muscle (CM) contraction in monkeys. Methods. Pupil diameter (vernier calipers) and refraction (coincidence refractometry) were measured every 15 minutes from 0.75 to 3.5 hours after topical LAT-A 42 mug (apprx10 muM in the anterior chamber (AC)). Refraction was measured every 5 minutes from 0.5 to 1.5 hours after intracameral injection of 10 mul of 50 muM LAT-A (apprx5 muM in AC), with intramuscular infusion of 1.5 mg/kg pilocarpine HCl (PILO) during the first 15 minutes of measurements. Pupil diameter was measured at 1 and 2 hours, and refraction was measured every 5 minutes from 1 to 2 hours, after intravitreal injection of 20 mul of 1.25 mM LAT-A (apprx10 muM in vitreous), with intramuscular infusion of 1.5 mg/kg PILO during the first 15 minutes of measurements (all after topical 2.5% phenylephrine), and contractile response of isolated CM strips, obtained <1 hour postmortem and mounted in a perfusion apparatus, to 10 muM PILO +/- LAT-A was measured at various concentrations. Results. Topical LAT-A of 42 mug dilated the pupil without affecting refraction. Intracameral LAT-A of 5 muM inhibited miotic and accommodative responses to intramuscular PILO. Intravitreal LAT-A of 10 muM had no effect on accommodative or miotic responses to intramuscular PILO. LAT-A dose-dependently relaxed the PILO-contracted CM by up to 50% at 3 muM in both the longitudinal and \*\*\*circular\*\*\* \*\*\*vectors\*\*\*. Conclusions. In monkeys, LAT-A causes mydriasis and cycloplegia, perhaps related to its known ability to disrupt the actin microfilament network and consequently to affect cell contractility and adhesion. Effects of LAT-A on the iris and CM may have significant physiological and clinical implications.

L2 ANSWER 5 OF 42 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1999:538505 CAPLUS

TITLE: Genetic Manipulation of Kinetoplastida

AUTHOR(S): Clayton, C. E.

CORPORATE SOURCE: Zentrum fur Molekulare Biologie (ZMBH), Heidelberg, D-69120, Germany

SOURCE: Parasitol. Today (1999), 15(9), 372-378  
CODEN: PATOE2; ISSN: 0169-4758

PUBLISHER: Elsevier Science Ltd.

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review with several refs. During the 1980s, many kinetoplastid genes were cloned and their function inferred from homol. with genes from other organisms, location of the corresponding proteins or expression in heterologous systems. Up until 1990, before the availability of DNA transfection methodol., the authors could not analyze the function of kinetoplastid genes within the organisms themselves. Since then, it has become possible to create and complement mutants, to overexpress foreign proteins in the parasites, to knock out genes and even to switch off essential functions. However, these methods are not equally applicable in all parasites. Here, Christine Clayton highlights the differences and

similarities between the most commonly used model organisms, and assesses the relative advantages of different approaches and parasites for different types of investigation. A breakthrough in transfection technol. started with the transient expression of reporter genes after electroporation of parasites with \*\*\*circular\*\*\* \*\*\*vectors\*\*\* and has since developed to include a wide spectrum of methods for functional gene anal.

L2 ANSWER 6 OF 42 MEDLINE

ACCESSION NUMBER: 1999377299 MEDLINE

DOCUMENT NUMBER: 99377299

TITLE: Use of a linear multicopy vector based on the mini-replicon of temperate coliphage N15 for cloning DNA with abnormal secondary structures.

AUTHOR: Ravin N V; Ravin V K

CORPORATE SOURCE: Bioengineering Center, Russian Academy of Sciences, Prospekt 60 let Oktiabria, bld.7-1, 117312 Moscow, Russia.

SOURCE: Nucleic Acids Res, (1999 Sep 1) 27 (17) e13.

Journal code: DFl. ISSN: 1362-4962.

PUB. COUNTRY: ENGLAND: United Kingdom

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199911

ENTRY WEEK: 19991101

AB A new cloning vector pN15L is described. It is a linear 13.8 kb plasmid based on the coliphage N15 mini-replicon. The vector capacity exceeds 50 kb and the copy number is 250 per Escherichia coli chromosome. We show that some artificial and natural palindromes and approximately 5% of human DNA Bgl II fragments can be cloned effectively in linear vector pN15L, whereas they either sharply reduce the copy number of \*\*\*circular\*\*\* \*\*\*vector\*\*\* pUC19 or cannot be cloned at all. We conclude that pN15L may be usefully employed to clone large imperfect palindromes and some abnormal sequences of human DNA.

L2 ANSWER 7 OF 42 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1998:71230 CAPLUS

DOCUMENT NUMBER: 128:137177

TITLE: Recombinant DNA vector comprising genomic equine arteritis virus sequences

INVENTOR(S): Spaan, Wilhelmus Josephus Maria; Bredenbeek, Petrus Johannes; Den Boon, Johan Arie; Van Dinten, Leonie Christina; Wassenaar, Alfred Leonard Maria; Snijder, Eric John

PATENT ASSIGNEE(S): Rijksuniversiteit Leiden, Neth.; Spaan, Wilhelmus Josephus Maria; Bredenbeek, Petrus Johannes; Den Boon, Johan Arie; Van Dinten, Leonie Christina; Wassenaar, Alfred Leonard Maria; Snijder, Eric John

SOURCE: PCT Int. Appl., 36 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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WO 9802549 A1 19980122 WO 1997-NL408 19970714  
W: AU, CA, CN, JP, US  
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE  
NL 1003579 C2 19980115 NL 1996-1003579 19960712  
AU 9733624 A1 19980209 AU 1997-33624 19970714  
EP 912744 A1 19990506 EP 1997-929604 19970714  
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,  
IE, FI

PRIORITY APPLN. INFO.:

NL 1996-1003579 19960712  
WO 1997-N

L408 19970714

AB The present invention relates to a recombinant DNA vector comprising a cDNA copy of at least a part of the RNA genome of equine arteritis virus. The viral cDNA was 5' linked to the T7 promoter, the 5' end was extended with a sequence comprising unique restriction sites for MdeI and NotI, and the 3' end was extended with a sequence coding for a unique XhoI restriction site. The construct is introduced in the vector pUC18 between the unique EcoRI and HindIII restriction sites, and these sites were cut and made blunt to allow ligation to yield the \*\*\*circular\*\*\*  
\*\*\*vector\*\*\* pEAV030. The recombinant DNA vector according to the present invention is stable in a vector host cell and yields, after transcription, infectious RNA. Due to the invention the genetic engineering of the virus is very much simplified and it may be used for, among other things, the prepn. of pharmaceutical compns.

L2 ANSWER 8 OF 42 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1998:499097 CAPLUS

DOCUMENT NUMBER: 129:91393

TITLE: Eliminating specific sequences from sequence libraries using RecA protein to prevent methylation

INVENTOR(S): Nahas, Nasri; Dumas, Milne Edwards Jean Baptiste

PATENT ASSIGNEE(S): Genset S. A., Fr.

SOURCE: Fr. Demande, 27 pp.

CODEN: FRXXBI

DOCUMENT TYPE: Patent

LANGUAGE: French

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
FR 2757537	A1	19980626	FR 1996-15854	19961223
FR 2757537	B1	19990319		
WO 9828439	A1	19980702	WO 1997-FR2378	19971222

W: CA, JP, US

RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

EP 948650 A1 19991013 EP 1997-952971 19971222

R: CH, DE, FR, GB, IT, LI, NL, SE

PRIORITY APPLN. INFO.:

FR 1996-15854 19961223

WO 1997-FR2378 19971222

AB A method of selectively eliminating specific sequences from a clone bank is described. This allows the removal from cDNA banks of sequences that are common to several tissues, allowing rapid identification of tissue-specific sequences. The method uses oligonucleotides derived from the sequence to be deleted and RecA protein. The clone bank is incubated with the oligonucleotides and the protein until a stable triple helix is formed and the DNA is then methylated. The triple-helical region is not

methyated so, when the complex is broken down, the protected region is susceptible to cleavage by a methylation-sensitive restriction endonuclease. If the bank is prep'd. in a closed \*\*\*circular\*\*\*  
\*\*\*vector\*\*\*, i.e. a plasmid, the linear DNA can then be further degraded with an exonuclease.

L2 ANSWER 9 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 3  
ACCESSION NUMBER: 1999:3174 BIOSIS  
DOCUMENT NUMBER: PREV199900003174  
TITLE: Geometry of \*\*\*circular\*\*\* \*\*\*vectors\*\*\* and  
pattern recognition of shape of a boundary.  
AUTHOR(S): Rao, Calyampudi R. (1)  
CORPORATE SOURCE: (1) Statistics Dep., 326 Joab Thomas Build., Pennsylvania  
State Univ., University Park, PA 16802 USA  
SOURCE: Proceedings of the National Academy of Sciences of the  
United States of America, (Oct. 27, 1998) Vol. 95, No. 22,  
pp. 12783-12786.  
ISSN: 0027-8424.  
DOCUMENT TYPE: Article  
LANGUAGE: English

AB This paper deals with pattern recognition of the shape of the boundary of closed figures on the basis of a circular sequence of measurements taken on the boundary at equal intervals of a suitably chosen argument with an arbitrary starting point. A distance measure between two boundaries is defined in such a way that it has zero value when the associated sequences of measurements coincide by shifting the starting point of one of the sequences. Such a distance measure, which is invariant to the starting point of the sequence of measurements, is used in identification or discrimination by the shape of the boundary of a closed figure. The mean shape of a given set of closed figures is defined, and tests of significance of differences in mean shape between populations are proposed.

L2 ANSWER 10 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 4  
ACCESSION NUMBER: 1998:480006 BIOSIS  
DOCUMENT NUMBER: PREV199800480006  
TITLE: Cre/lox-mediated site-specific integration of Agrobacterium  
T-DNA in Arabidopsis thaliana by transient expression of  
cre.  
AUTHOR(S): Vergunst, Annette C. (1); Hooykaas, Paul J. J.  
CORPORATE SOURCE: (1) Inst. Mol. Plant Sci., Wassenaarseweg 64, 2333 AL  
Leiden Netherlands  
SOURCE: Plant Molecular Biology, (Oct., 1998) Vol. 38, No. 3, pp.  
393-406.  
ISSN: 0167-4412.  
DOCUMENT TYPE: Article  
LANGUAGE: English

AB The Cre/lox system was used to obtain targeted integration of an Agrobacterium T-DNA at a lox site in the genome of Arabidopsis thaliana. Site-specific recombinants, and not random events, were preferentially selected by activation of a silent lox-neomycin phosphotransferase (nptII) target gene. To analyse the effectiveness of Agrobacterium-mediated transfer we used T-DNA vectors harbouring a single lox sequence (this vector had to circularize at the T-DNA left- and right-border sequences prior to site-specific integration) or two lox sequences (this vector allowed circularization at the lox sequences within the T-DNA either prior to or after random integration, followed by targeting of the

\*\*\*circularized\*\*\* \*\*\*vector\*\*\* ), respectively. Furthermore, to control the reversibility of the integration reaction, Cre recombinase was provided transiently by using a cotransformation approach. One precise stable integrant was found amongst the recombinant calli obtained after transformation with a double-lox T-DNA vector. The results indicate that Agrobacterium-mediated transformation can be used as a tool to obtain site-specific integration.

L2 ANSWER 11 OF 42 SCISEARCH COPYRIGHT 2000 ISI (R) DUPLICATE 5

ACCESSION NUMBER: 1998:162198 SCISEARCH

THE GENUINE ARTICLE: YX565

TITLE: Cloning of large imperfect palindromes in circular and linear vectors

AUTHOR: Ravin N V (Reprint); Ravin V K

CORPORATE SOURCE: RUSSIAN ACAD SCI, CTR BIOENGN, MOSCOW 177312, RUSSIA (Reprint)

COUNTRY OF AUTHOR: RUSSIA

SOURCE: GENETIKA, (JAN 1998) Vol. 34, No. 1, pp. 38-44.

Publisher: MEZHDUNARODNAYA KNIGA, 39 DIMITROVA UL., 113095

MOSCOW, RUSSIA.

ISSN: 0016-6758.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: Russian

REFERENCE COUNT: 21

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Data on comparative analysis of cloning of large imperfect artificial palindromes and one natural palindrome in the circular pUC19 and linear pN15L vectors, constructed on the basis of temperate N15 bacteriophage minireplicon are presented. The artificial palindromes consisted of the two head-to-head oriented 5:5-kb lambda bacteriophage DNA fragments interrupted by a short sequence of varied size. Natural palindrome was represented by the 12.5-kb BamHI fragment of Tetrahymena pyriformis ribosomal genes cluster. Integration of some artificial palindromes and a natural palindrome into a \*\*\*circular\*\*\* \*\*\*vector\*\*\* resulted in a considerable decrease of its copy number. This was assumed to result from cruciform formation mediated by supercoiling of a \*\*\*circular\*\*\* \*\*\*vector\*\*\* DNA. Thus, a linear is vector preferable in cloning of inverted repeated DNA sequences.

L2 ANSWER 12 OF 42 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1998:115015 CAPLUS

DOCUMENT NUMBER: 128:240059

TITLE: Cloning of large imperfect palindromes in circular and linear vectors

AUTHOR(S): Ravin, N. V.; Ravin, V. K.

CORPORATE SOURCE: Bioengineering Center, Russian Academy of Sciences, Moscow, 117312, Russia

SOURCE: Russ. J. Genet. (1998), 34(1), 31-36

CODEN: RJGEEQ; ISSN: 1022-7954

PUBLISHER: MAIK Nauka/Interperiodica Publishing

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Data on comparative anal. of cloning of large imperfect artificial palindromes and one natural palindrome in the circular pUC19 and linear pN15L vectors, constructed on the basis of temperate N15 bacteriophage minireplicon, are presented. The artificial palindromes consisted of the

two head-to-head oriented 5.5-kb lambda bacteriophage DNA fragments interrupted by a short sequence of varied size. Natural palindrome was represented by the 12.5-kb BamHI fragment of Tetrahymena pyriformis ribosomal genes cluster. Integration of certain artificial palindromes and a natural palindrome into a \*\*\*circular\*\*\* \*\*\*vector\*\*\* resulted in a considerable decrease of its copy no. This was assumed to result from cruciform formation mediated by supercoiling of a \*\*\*circular\*\*\* \*\*\*vector\*\*\* DNA. Thus, a linear vector is preferable in cloning of inverted repeated DNA sequences.

L2 ANSWER 13 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS

ACCESSION NUMBER: 1998:5914 BIOSIS

DOCUMENT NUMBER: PREV199800005914

TITLE: Circular YAC vectors containing a small mammalian origin sequence can associate with the nuclear matrix.

AUTHOR(S): Cossons, N.; Nielsen, T. O.; Dini, C.; Tomilin, N.; Young, D. B.; Riabowol, K. T.; Rattner, J. B.; Johnston, R. N.; Zannis-Hadjopoulos, M.; Price, G. B. (1)

CORPORATE SOURCE: (1) McGill Cancer Cent., 3655 Drummond Street, Montreal, PQ H3G 1Y6 Canada

SOURCE: Journal of Cellular Biochemistry, (Dec. 15, 1997) Vol. 67, No. 4, pp. 439-450.  
ISSN: 0730-2312.

DOCUMENT TYPE: Article

LANGUAGE: English

AB Three different mammalian origins of DNA replication, 343, S3, and X24, have been cloned into a 1 5.8 kb circular yeast vector pYACneo. Subsequent transfection into HeLa cells resulted in the isolation of several stably maintained clones. Two cell lines, C343e2 and CS3e1, were found to have sequences maintained as episomes in long-term culture with a stability per generation of approximately 80%. Both episomes also contain matrix attachment region (MAR) sequences which mediate the binding of DNA to the nuclear skeleton and are thought to play a role in DNA replication. Using high salt extraction of the nucleus and fluorescent in situ hybridization, we were able to demonstrate an association of the 343 episome with the nuclear matrix, most probably through functional MAR sequences that allow an association with the nuclear matrix and associated regions containing essential replication proteins. The presence of functional MARs in small episomal sequences may facilitate the replication and maintenance of transfected DNA as an episome and improve their utility as small episomal constructs, potential microchromosomes.

L2 ANSWER 14 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 6

ACCESSION NUMBER: 1997:454283 BIOSIS

DOCUMENT NUMBER: PREV199799753486

TITLE: Autonomous plasmid replication in Aspergillus nidulans: AMA1 and MATE elements.

AUTHOR(S): Aleksenko, A. (1); Clutterbuck, A. J.

CORPORATE SOURCE: (1) Inst. Genetics Selection Industrial Microorganisms, Moscow 113545 Russia

SOURCE: Fungal Genetics and Biology, (1997) Vol. 21, No. 3, pp. 373-387.  
ISSN: 1087-1845.

DOCUMENT TYPE: Article

LANGUAGE: English

AB With few exceptions, in eukaryotic organisms the presence of a chromosomal replicator on a \*\*\*circular\*\*\* \*\*\*vector\*\*\* molecule is not

sufficient to confer on it the ability to persist and replicate extrachromosomally. However, it is possible to isolate from genomes of some filamentous fungi DNA fragments which can provide extrachromosomal maintenance of plasmids. In *Aspergillus nidulans*, two functional classes of such sequences can be distinguished: effective plasmid replicators (e.g., AMAl) and transformation enhancers (e.g., ANS1 or MATEs), which apparently are able to initiate aberrant replication, leading to vector rearrangement and multimerization and eventually resulting in chromosomal integration. We discuss the similarity of these events to DNA amplification in other eukaryotes. A model is suggested which accounts for the formation of effective replicating plasmids as a result of sequence amplification. The model is based on the observation that in some organisms, including *A. nidulans* and *Schizosaccharomyces pombe*, duplication of an inefficient replicator enhances its efficiency dramatically. Some structural traits of transformation enhancers in *A. nidulans* imply a role for topoisomerases in amplification and replication of circular DNA molecules. We discuss practical applications of replicative vectors for gene cloning and expression studies.

L2 ANSWER 15 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 7  
 ACCESSION NUMBER: 1997:150372 BIOSIS  
 DOCUMENT NUMBER: PREV199799449575  
 TITLE: Generation of catalytic RNAs by rolling transcription of synthetic DNA nanocircles.  
 AUTHOR(S): Daubendiek, Sarah L.; Kool, Eric T. (1)  
 CORPORATE SOURCE: (1) Dep. Biochem. Biophysics, Univ. Rochester, Rochester, NY 14627 USA  
 SOURCE: Nature Biotechnology, (1997) Vol. 15, No. 3, pp. 273-277. ISSN: 1087-0156.  
 DOCUMENT TYPE: Article  
 LANGUAGE: English

AB Small catalytic RNAs are commonly produced either by transcription of promoter-driven linear DNA templates or by stepwise chemical synthesis on solid supports. We describe a different approach, in which very small chemically synthesized circular DNAs serve as efficient templates for generation of catalytic RNAs in vitro. The circles are 83 nucleotides in size, are single stranded, and contain no canonical RNA polymerase promoters. Despite this, T7 and *Escherichia coli* RNA polymerases transcribe the circles by a rolling mechanism, producing long concatemeric RNAs (apprx 7,500 nt). During the transcription reaction, the repeating RNAs self-cleave, ultimately reaching monomer length. Despite having self-complementary sequences at their substrate-binding domains, these monomeric 83-nt RNAs are shown to be catalytically active ribozymes that sequence-specifically cleave RNA targets in trans. In addition, a \*\*\*circular\*\*\* \*\*\*vector\*\*\* encoding a repeating (non-self-processing) ribozyme is described; the resulting multimeric ribozyme, targeted to a sequence in the HIV-1 genome, is also catalytically active in trans. This novel approach to the synthesis of catalytic RNAs offers a number of differences and potential advantages over current approaches to RNA synthesis.

L2 ANSWER 16 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 8  
 ACCESSION NUMBER: 1997:486917 BIOSIS  
 DOCUMENT NUMBER: PREV199799786120  
 TITLE: Transfer of small YACs to *E. coli* as large circular plasmids.  
 AUTHOR(S): Frengen, Eirik; Wu, Chenyan; De Jong, Pieter J. (1)

CORPORATE SOURCE: (1) Dep. Human Genetics, Roswell Park Cancer Inst., Elm and  
Carlton Streets, Buffalo, NY 14263 USA  
SOURCE: Genetic Analysis Biomolecular Engineering, (1997) Vol. 14,  
No. 2, pp. 55-59.

DOCUMENT TYPE: Article

LANGUAGE: English

AB We have designed a YAC \*\*\*circularization\*\*\* \*\*\*vector\*\*\*, pCIRC3,  
allowing enrichment of the YAC DNA by exonuclease digestion of the linear  
yeast chromosomes. Due to the presence of Pl replicon sequences in this  
vector, the circular YACs would replicate as PACs in Escherichia coli.

L2 ANSWER 17 OF 42 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1996:161348 CAPLUS

DOCUMENT NUMBER: 124:194354

TITLE: Method for gene therapy using DNA vector lacking a  
selection marker gene

INVENTOR(S): Seeber, Stefan; Rueger, Ruediger

PATENT ASSIGNEE(S): Boehringer Mannheim GmbH, Germany

SOURCE: Ger. Offen., 8 pp.

CODEN: GWXXBX

DOCUMENT TYPE: Patent

LANGUAGE: German

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 4428402	A1	19960215	DE 1994-4428402	19940811
WO 9605297	A1	19960222	WO 1995-EP3027	19950731
W: CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2197075	AA	19960222	CA 1995-2197075	19950731
EP 775203	A1	19970528	EP 1995-929028	19950731
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
JP 09512030	T2	19971202	JP 1995-506966	19950731
PRIORITY APPLN. INFO.:				DE 1994-4428402 19940811
				WO 1995-EP3027 19950731

AB \*\*\*Circular\*\*\* \*\*\*vector\*\*\* DNA contg. a selection marker gene and  
a heterologous sequence to be introduced into cells for therapeutic  
purposes is amplified under selective pressure and subsequently cleaved so  
that the selection marker and the heterologous DNA are on sep. DNA  
fragments. The fragments are recircularized and are sepd. before or after  
recircularization, and the recircularized DNA contg. the heterologous  
sequence, which now lacks the selection marker, is introduced into cells  
by transfection. This procedure avoids transfer of e.g. antibiotic  
resistance genes to bacteria of the human lung and intestine during gene  
therapy. Thus, a 5.8-kb DNA fragment contg. the therapeutic CMV-CFTR gene  
cassette was inserted into a pUC18-derived plasmid contg. an ampicillin  
resistance gene and a polylinker, such that the circular construct could  
be cleaved with PvuI, followed by PvuI + PstI, into a 5.8-kb fragment and  
several smaller fragments which were religated with T4 ligase and sepd. by  
mol. sieve chromatog.

L2 ANSWER 18 OF 42 SCISEARCH COPYRIGHT 2000 ISI (R)

ACCESSION NUMBER: 96:309672 SCISEARCH

THE GENUINE ARTICLE: UF005

TITLE: A COMPARISON OF HOLOCENE PALEOMAGNETIC SECULAR VARIATION

RECORDS FROM NORTH-AMERICA  
AUTHOR: LUND S P (Reprint)  
CORPORATE SOURCE: UNIV SO CALIF, DEPT EARTH SCI, UNIV PK, LOS ANGELES, CA,  
90089 (Reprint)  
COUNTRY OF AUTHOR: USA  
SOURCE: JOURNAL OF GEOPHYSICAL RESEARCH-SOLID EARTH, (10 APR 1996)  
Vol. 101, No. B4, pp. 8007-8024.  
ISSN: 0148-0227.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: PHYS  
LANGUAGE: ENGLISH  
REFERENCE COUNT: 41

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The comparison of nine Holocene (0-12,000 years B.P.) records of paleomagnetic field secular variation (PSV) obtained from across North America indicates that distinctive field features in inclination and declination often can be traced for more than 4000 km without significant change in pattern. Independent age determinations corroborate these correlations while also suggesting that westward (or eastward) drift is not significant. On the other hand, northward drift may be a significant process at least between 1500 and 5500 years B.P. Several methods of analyzing the overall PSV character point to \*\*\*circularity\*\*\* ( \*\*\*vector\*\*\* looping) as a preferred means of defining coherent space-time vector variations which are termed 'waveforms'. A few distinctive waveforms, each discernible for about 1400 years, dominate PSV across North America during the Holocene; 4.5 loops and 2 loops being associated with clockwise and counterclockwise circularity, respectively. The clockwise loops appear to be periodic, occurring every 2400 years, and most likely result from the recurrence of a distinctive regional dynamo source in the outer core. The pattern of circularity noted in North America during the last 3000 years correlates in time with similar circularity changes seen globally. This may indicate that there is some degree of coupling between different regional dynamo sources within the outer core. The fact that some North American waveforms are almost identical to waveforms observed elsewhere around the world at different times may indicate that similar distinctive regional dynamo sources recur randomly throughout the outer core.

L2 ANSWER 19 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 9

ACCESSION NUMBER: 1994:295843 BIOSIS  
DOCUMENT NUMBER: PREV199497308843  
TITLE: A method for linking V-L and V-H region genes that allows bulk transfer between vectors for use in generating polyclonal IgG libraries.  
AUTHOR(S): Sarantopoulos, Stefanie; Kao, Chiou-Ying Y.; Den, Wen; Sharon, Jacqueline (1)  
CORPORATE SOURCE: (1) Boston Univ. Sch. Med., 80 East Concord St., K707, Boston, MA 02118 USA  
SOURCE: Journal of Immunology, (1994) Vol. 152, No. 11, pp. 5344-5351.  
ISSN: 0022-1767.  
DOCUMENT TYPE: Article  
LANGUAGE: English

AB Libraries of Ab fragments have been produced by others from light and heavy chain cDNAs derived from populations of B lymphocytes and expressed in bacteria. However, such libraries have not been transferred to eukaryotic expression vectors to generate polyclonal libraries of intact

glycosylated Abs that can mediate effector functions. We present a method for transferring pairs of linked V-L-V-H region genes between circular prokaryotic and eukaryotic vectors. The key feature of the transfer is that the V-L and V-H region genes are linked head to head ( tautm ) in opposite transcriptional orientations. To illustrate this method, a pair of V-L and V-H region cDNAs derived from an existing hybridoma cell line were linked head to head by PCR, transferred as a unit between vectors, and expressed as an IgG Ab with Ag binding activity. Although we tested the transfer of a single V-L-V-H region gene pair, this system is expected to allow the bulk transfer of physically linked V-L-V-H region gene combinations between different \*\*\*circular\*\*\* \*\*\*vectors\*\*\* and the expression of the same library as either Ab fragments or intact Abs.

L2 ANSWER 20 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 10

ACCESSION NUMBER: 1995:33551 BIOSIS

DOCUMENT NUMBER: PREV199598047851

TITLE: High-efficiency transformation of *Pichia stipitis* based on its URA3 gene and a homologous autonomous replication sequence, ARS2.

AUTHOR(S): Yang, Vina W.; Marks, Jere A.; Davis, Brian P.; Jeffries, Thomas W. (1)

CORPORATE SOURCE: (1) Forest Products Lab., One Gifford Pinchot Drive, Madison, WI 53705 USA

SOURCE: Applied and Environmental Microbiology, (1994) Vol. 60, No. 12, pp. 4245-4254.  
ISSN: 0099-2240.

DOCUMENT TYPE: Article

LANGUAGE: English

AB This paper describes the first high-efficiency transformation system for the xylose-fermenting yeast *Pichia stipitis*. The system includes integrating and autonomously replicating plasmids based on the gene for orotidine-5'-phosphate decarboxylase (URA3) and an autonomous replicating sequence (ARS) element (ARS2) isolated from *P. stipitis* CBS 6054. Ura-auxotrophs were obtained by selecting for resistance to 5-fluoroorotic acid and were identified as *ura3* mutants by transformation with *P. stipitis* URA3. *P. stipitis* URA3 was cloned by its homology to *Saccharomyces cerevisiae* URA3, with which it is 69% identical in the coding region. *P. stipitis* ARS elements were cloned functionally through plasmid rescue. These sequences confer autonomous replication when cloned into vectors bearing the *P. stipitis* URA3 gene. *P. stipitis* ARS2 has features similar to those of the consensus ARS of *S. cerevisiae* and other ARS elements. Circular plasmids bearing the *P. stipitis* URA3 gene with various amounts of flanking sequences produced 600 to 8,600 Ura+ transformants per  $\mu$ -g of DNA by electroporation. Most transformants obtained with \*\*\*circular\*\*\* \*\*\*vectors\*\*\* arose without integration of vector sequences. One vector yielded 5,200 to 12,500 Ura+ transformants per  $\mu$ -g of DNA after it was linearized at various restriction enzyme sites within the *P. stipitis* URA3 insert. Transformants arising from linearized vectors produced stable integrants, and integration events were site specific for the genomic *ura3* in 20% of the transformants examined. Plasmids bearing the *P. stipitis* URA3 gene and ARS2 element produced more than 30,000 transformants per  $\mu$ -g of plasmid DNA. Autonomously replicating plasmids were stable for at least 50 generations in selection medium and were present at an average of 10 copies per nucleus.

L2 ANSWER 21 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 11

ACCESSION NUMBER: 1995:20857 BIOSIS  
DOCUMENT NUMBER: PREV199598035157  
TITLE: Development of a method for the vector transformation of the methylotrophic yeast *Pichia methanolica*.  
AUTHOR(S): Tarutina, M. G.; Tolstorukov, I. I.  
CORPORATE SOURCE: State Sci. Cent. Genet. Sel. Ind. Microorg., Moscow 113545 Russia  
SOURCE: Genetika, (1994) Vol. 30, No. 6, pp. 783-790.  
ISSN: 0016-6758.  
DOCUMENT TYPE: Article  
LANGUAGE: Russian  
SUMMARY LANGUAGE: Russian; English

AB A method for transformation of the methylotrophic yeast *Pichia methanolica* (formerly *P. pinus* MH4) was developed. Mutants *leu1* were shown to be transformed with different efficiency using 2.2-10.7-kb linear and circular DNA molecules containing the *LEU2* gene of the yeast *Saccharomyces cerevisiae*, which complemented the *leu1* mutation of the recipient. Efficiency of transformation with short molecules was higher than with long molecules. Transformation with linear DNA was more efficient than with circular DNA of equal size. Transformants contained both replication-unstable and integration forms in different proportions. Significant rearrangements in the episome and integration forms of transforming DNA were found in transformants obtained using the 10.7-kb circular YEp13 plasmid. Integration of linear DNA was not accompanied by rearrangements in DNA molecules. Certain clones isolated after transformation with linear DNA contained autonomously replicating \*\*\*circular\*\*\* \*\*\*vector\*\*\* molecules formed as a result of a ligase reaction in vivo. The *LEU2* gene of *S. cerevisiae* contains an unknown sequence acting as the *ARS* replicon in cells of both *P. methanolica* and of another species of the methylotrophic yeasts, *Hansenula polymorpha*.

L2 ANSWER 22 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 12

ACCESSION NUMBER: 1995:110546 BIOSIS  
DOCUMENT NUMBER: PREV199598124846  
TITLE: The effect of muscarinic agonists and selective receptor subtype antagonists on the contractile response of the isolated rhesus monkey ciliary muscle.  
AUTHOR(S): Poyer, John F.; Gabelt, B'Ann T.; Kaufman, Paul L. (1)  
CORPORATE SOURCE: (1) Dep. Ophthalmol., Univ. Wisconsin, Clin. Sci. Cent., 600 Highland Ave., Madison, WI 53792-3220 USA  
SOURCE: Experimental Eye Research, (1994) Vol. 59, No. 6, pp. 729-736.  
ISSN: 0014-4835.  
DOCUMENT TYPE: Article  
LANGUAGE: English

AB There are ultrastructural and histochemical differences between the longitudinal (putatively more relevant to outflow facility) and circular (putatively more relevant to accommodation) portions of the primate ciliary muscle. Oxotremorine, a muscarinic agonist putatively somewhat selective for the M-2 receptor subtype, binds preferentially to the longitudinal rather than the circular portion. Aceclidine, a putatively non-subtype selective muscarinic agonist, can dissociate accommodative and outflow facility responses in monkeys and humans. We used the muscarinic receptor subtype antagonists 4-diphenylacetoxy-N-methylpiperidine methobromide (4-DAMP), 11-((2-(diethylamino)methyl)-1-piperidinyloxy)acetyl-5,11-dihydro-6H-pyrido(2,3b)(1,4)benzodiazepine-6-one (AF-DX 116), and

pirenzepine to inhibit contractile responses to the muscarinic agonists carbachol, aceclidine and oxotremorine in the longitudinal and

\*\*\*circular\*\*\* \*\*\*vectors\*\*\* of the rhesus monkey ciliary muscle in vitro. Oxotremorine generated dose-response curves that were similar in both the circular and longitudinal vectors and intermediate to those previously reported for carbachol and aceclidine. 4-DAMP (M-3 selective) was the most potent inhibitor of contractile responses to all three agonists, with IC-50 values ranging from 33 to 68 nM for the circular and from 27 to 63 nM for the longitudinal vector, depending on the agonist used to elicit contraction. Pirenzepine (M-1 selective) was 25-fold less potent and AF-DX 116 (M-2 selective) was 108-fold less potent at inhibiting contractile responses to all three agonists in either vector, indicating that M-3 is the predominant receptor subtype mediating ciliary muscle contraction in both vectors. The IC-25, IC-50 and IC-75 values for all three antagonists against aceclidine was approximately 1.4-fold less for the longitudinal than the \*\*\*circular\*\*\*

\*\*\*vector\*\*\*, of uncertain relevance to aceclidine's in vivo facility-accommodation dissociation.

L2 ANSWER 23 OF 42 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1992:188774 CAPLUS

DOCUMENT NUMBER: 116:188774

TITLE: Use of oligonucleotides and nick translation for site-directed mutagenesis in plasmids

AUTHOR(S): Drutsa, V. L.; Kaberdin, V. R.

CORPORATE SOURCE: A. N. Belozerskii Inst. Phys. Chem. Biol., Moscow State Univ., Moscow, 119899, USSR

SOURCE: Nucleic Acids Res. (1992), 20(4), 922

CODEN: NARHAD; ISSN: 0305-1048

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A new, effective and rather general approach was used for

oligonucleotide-directed site-specific mutagenesis and cloning of single-stranded DNA fragments in double-stranded \*\*\*circular\*\*\*

\*\*\*vectors\*\*\* without using specially constructed host-vector systems. The main stages of the target mutant plasmid construction are outlined in the scheme proposed. The first stage involves cleavage of original circular DNA at a chosen unique restriction site and treatment of the digested DNA with exonuclease III to generate protruding single-stranded termini. Then the DNA prep. is annealed with synthetic primers one of which is a mutagenic oligonucleotide complementary (except for the bases to be changed) to the single-stranded region to be mutagenized. The other two are deca-octadecanucleotide adapters, perfectly complementary to the 5'-ends of the linearized vector. The structures of the 3'-ends of the adapters are chosen specially to regenerate the original restriction site upon annealing and DNA ligase treatment. The mutagenic oligonucleotide and one of the adapters complementary to the same DNA strand must possess the 5'-phosphate groups. The second adapter, on the contrary, should have no end phosphates. The hybrid obtained is recircularized with DNA ligase, filled in with DNA polymerase, treated with DNA ligase, and a nick adjacent to the 5'-end of the nonphosphorylated adapter is moved through the region to be mutagenized by E. coli DNA polymerase I treatment. The DNA mixt. thus obtained is used directly for transformation of any competent cells. Following this procedure several target mutations (pt bp substitutions, deletions and insertions up to 67 bp length) were introduced in various plasmids with 64-95% yields.

L2 ANSWER 24 OF 42 SCISEARCH COPYRIGHT 2000 ISI (R)  
 ACCESSION NUMBER: 92:518063 SCISEARCH  
 THE GENUINE ARTICLE: JK884  
 TITLE: TYPES AND KINEMATIC STABILITY OF TRIPLE JUNCTIONS  
 AUTHOR: CRONIN V S (Reprint)  
 CORPORATE SOURCE: UNIV WISCONSIN, DEPT GEOSCI, POB 413, MILWAUKEE, WI, 53201  
 (Reprint)  
 COUNTRY OF AUTHOR: USA  
 SOURCE: TECTONOPHYSICS, (15 JUL 1992) Vol. 207, No. 3-4, pp.  
 287-301.  
 ISSN: 0040-1951.  
 DOCUMENT TYPE: Article; Journal  
 FILE SEGMENT: PHYS  
 LANGUAGE: ENGLISH  
 REFERENCE COUNT: 45

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB A triple junction is kinematically stable if the orientation of each plate boundary remains constant relative to other boundaries in the triple junction during a finite time-interval. Instantaneous relative velocity vectors have been used elsewhere to indicate the relative motion of plates during a finite time-interval - a technique that is not generally valid where finite relative plate motion is not circular. In the typical case in which all three plates have non-zero velocities around different plate-specific poles of rotation, the direction and magnitude of relative plate velocity varies systematically with time. Five plate-boundary types are considered in evaluating triple-junction stability: ridges (R), right-lateral and left-lateral transform faults (F(R) and F(L)), and trenches in which the overriding plate is clockwise (T(C)) or anticlockwise (T(A)) from the trench, judged by a rotation around the triple junction. One hundred twenty five triplet combinations of boundary types are possible. Some combinations display symmetry with other combinations. Grouping all similar configurations, 25 types of triple junctions can be distinguished, of which 19 types may be stable when finite relative motion is \*\*\*circular\*\*\* . \*\*\*Vectorial\*\*\* descriptors of the geometry of ridges, transform faults and trenches permit the listing of general conditions for the kinematic stability of triple junctions, under conditions of both circular and non-circular relative motion. The TJ1 model for the evolution of RRR triple junctions provides estimates of the variation in the geometry of triple junctions in which spreading is orthogonal and near-symmetric. RRR triple junctions at which spreading is orthogonal and symmetric are always stable when the finite relative motion of all corresponding plates is circular: however, TJ1 indicates that RRR triple junctions are not generally stable when relative motion is non-circular. In general, triple junctions are not kinematically stable, but evolve with changes in the finite relative motion of plates.

L2 ANSWER 25 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 13  
 ACCESSION NUMBER: 1991:341313 BIOSIS  
 DOCUMENT NUMBER: BA92:40688  
 TITLE: EFFECTS OF EXCESS CENTROMERES AND EXCESS TELOMERES ON  
 CHROMOSOME LOSS RATES.  
 AUTHOR(S): RUNGE K W; WELLINGER R J; ZAKIAN V Z  
 CORPORATE SOURCE: FRED HUTCHINSON CANCER RES. CENTER, DIV. BASIC SCI., M621,  
 1124 COLUMBIA ST., SEATTLE, WASH. 98104.  
 SOURCE: MOL CELL BIOL, (1991) 11 (6), 2919-2928.  
 CODEN: MCEBD4. ISSN: 0270-7306.

FILE SEGMENT: BA; OLD  
LANGUAGE: English

AB The linear chromosomes of eukaryotes contain specialized structures to ensure their faithful replication and segregation to daughter cells. Two of these structures, centromeres and telomeres, are limited, respectively, to one and two copies per chromosome. It is possible that the proteins that interact with centromere and telomere DNA sequences are present in limiting amounts and could be competed away from the chromosomal copies of these elements by additional copies introduced on plasmids. We have introduced excess centromeres and telomeres into *Saccharomyces cerevisiae* and quantitated their effects on the rates of loss of chromosome III and chromosome VII by fluctuation analysis. We show that (i) 600 new telomeres hve no effect on chromosome loss; (ii) an average of 25 extra centromere DNA sequences increase the rate of chromosome III loss from 0.4 .times. 10<sup>-4</sup> events per cell division to 1.3 .times. 10<sup>-3</sup> events per cell division; (iii) centromere DNA (CEN) sequences on \*\*\*circular\*\*\* \*\*\*vectors\*\*\* destabilize chromosomes more effectively than do CEN sequences on 15-kb linear vectors, and transcribed CEN sequences have no effect on chromosome stbility. We discuss the different effects of extra centromere and telomere DNA sequences on chromosome stability in terms of how the cell recognizes these two chromosomal structures.

L2 ANSWER 26 OF 42 SCISEARCH COPYRIGHT 2000 ISI (R)  
ACCESSION NUMBER: 91:592024 SCISEARCH  
THE GENUINE ARTICLE: GL713  
TITLE: A MICROSTRIP LINE ON A CHIRAL SUBSTRATE  
AUTHOR: KLUSKENS M S (Reprint); NEWMAN E H  
CORPORATE SOURCE: OHIO STATE UNIV, DEPT ELECT ENGN, ELECTROSCI LAB, 1320 KINNEAR RD, COLUMBUS, OH, 43210 (Reprint)  
COUNTRY OF AUTHOR: USA  
SOURCE: IEEE TRANSACTIONS ON MICROWAVE THEORY AND TECHNIQUES, (1991) Vol. 39, No. 11, pp. 1889-1891.  
DOCUMENT TYPE: Note; Journal  
FILE SEGMENT: ENGI  
LANGUAGE: ENGLISH  
REFERENCE COUNT: 15

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Right and left \*\*\*circular\*\*\* \*\*\*vector\*\*\* potentials are developed and used in a spectral-domain solution for a microstrip transmission line on a chiral substrate. These vector potentials have properties similar to those of the usual magnetic and electric vector potentials, except that they result in circular rather than linearly polarized fields, thereby simplifying field expansions in chiral media. The chiral microstrip line does not have bifurcated modes like other chiral guided wave structures; however, the chiral substrate causes a significant asymmetry in both the fields and currents.

L2 ANSWER 27 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 14  
ACCESSION NUMBER: 1992:72331 BIOSIS  
DOCUMENT NUMBER: BA93:40786  
TITLE: PROMOTER ACTIVITY ASSOCIATED WITH THE LEFT INVERTED TERMINAL REPEAT OF THE KILLER PLASMID K1 FROM YEAST.  
AUTHOR(S): CHEN X J; WESOLOWSKI-LOUVEL M; TANGUY-ROUGEAU C; FUKUHARA H  
CORPORATE SOURCE: INST. CURIE, SECT. BIOL., BAT 110, CENT. UNIVERSITAIRE, 91405 ORSAY, FRANCE.  
SOURCE: BIOCHIMIE (PARIS), (1991) 73 (9), 1195-1204.  
CODEN: BICMBE. ISSN: 0300-9084.

FILE SEGMENT: BA; OLD  
LANGUAGE: English

AB The killer plasmid k1 of Kluyveromyces lactis has terminal inverted repeats of 202 base pairs (bp). The left terminal repeat is contiguous to the transcribed open reading frame, ORFI, which is supposed to code for a DNA polymerase. A 226-bp fragment (called Pk1) containing most of the terminal repeat sequence was isolated and examined for promoter activity. Pk1 was fused, in either original or inversed orientation, with a promoter-less lacZ gene of Escherichia coli and a promoter-less G418 resistance gene of Tn903. These fusions were introduced into a pKD1-derived \*\*\*circular\*\*\* \*\*\*vector\*\*\*, and transformed into a lactose-negative (lac4), and a G418-sensitive K. lactis host. Lac+ and G418-resistant transformants were obtained with either orientation or Pk1. The promoter activity of Pk1 fragment was independent of the presence or absence of killer plasmids. It is now known whether P, can also function bidirectionally on the natural k1 plasmid. The possible functions of Pk1 for killer plasmid gene expression and plasmid replication are discussed.

L2 ANSWER 28 OF 42 MEDLINE

ACCESSION NUMBER: 93027406 MEDLINE  
DOCUMENT NUMBER: 93027406  
TITLE: General method for site-directed mutagenesis and cloning of synthetic single-stranded DNA in \*\*\*circular\*\*\*  
\*\*\*vectors\*\*\*.  
AUTHOR: Drutsa V L; Kaberdin V R  
CORPORATE SOURCE: A.N. Belozersky Laboratory, Moscow State University, USSR.  
SOURCE: NUCLEIC ACIDS SYMPOSIUM SERIES, (1991) (24) 300.  
Journal code: O8N. ISSN: 0261-3166.  
PUB. COUNTRY: ENGLAND: United Kingdom  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199301

L2 ANSWER 29 OF 42 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1991:600360 CAPLUS  
DOCUMENT NUMBER: 115:200360  
TITLE: In vitro system for isolation and manipulation of DNA sequences with limited homology  
INVENTOR(S): Resnick, M.  
PATENT ASSIGNEE(S): National Institutes of Health, USA  
SOURCE: U. S. Pat. Appl., 17 pp. Avail. NTIS Order No.  
PAT-APPL-6-457 557.  
CODEN: XAXXAV  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 457557	A0	19901215	US 1989-457557	19891227
WO 9109954	A1	19910711	WO 1990-US7540	19901212
W: AU, BB, BG, BR, FI, HU, JP, KP, KR, LK, MC, MG, MW, NL, NO, RO, SD, SU				
RW: AT, BE, BF, BJ, CF, CG, CH, CM, DE, DK, ES, FR, GA, GR, IT, LU, ML, MR, NL, SE, SN, TD, TG				

AU 9171627	A1	19910724	AU 1991-71627	19901212
CA 2033116	AA	19910628	CA 1990-2033116	19901224
GB 2239456	A1	19910703	GB 1990-28107	19901227
GB 2239456	B2	19940525		
US 5334522	A	19940802	US 1992-860233	19920327

PRIORITY APPLN. INFO.:

US 1989-457557	19891227
WO 1990-US7540	19901212

AB The title in vitro system comprises two cloning vectors having the same backbone, but one contains a DNA probe sequence and the other contains the test DNA. On one end of the DNA probe there is a first unique restriction site; on the other end of the test DNA there is a second unique restriction site. The method comprises mixing the vectors (probe DNA:test DNA = 10:1) nicked with the unique restriction enzymes, denaturing, renaturing first at high stringency conditions (to hybridize complementary plasmid sequences) and then at low stringency conditions (to hybridize probe with test DNA). An appropriate mismatch repair mutant is transformed with the resulting mixt. of linear and circular DNA mols. This allows replication of \*\*\*circular\*\*\* \*\*\*vectors\*\*\* (contg. hybridized probe and test DNA) but not linear vectors (contg. only probe DNA), and selection of colonies contg. the DNA of interest. Preferably, a marker gene such as lacZ is included in the cloning vector contg. the test DNA. The method also enables the prepn. of modified genes when a host such as E. coli mutU mutants, which have a very short patch mismatch repair system, are used.

L2 ANSWER 30 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 15

ACCESSION NUMBER: 1991:135949 BIOSIS

DOCUMENT NUMBER: BA91:72489

TITLE: SITE-DIRECTED RECOMBINATION-MEDIATED MUTAGENESIS OF A COMPLEX GENE LOCUS.

AUTHOR(S): BARTON M C; HOEKSTRA M F; EMERSON B M

CORPORATE SOURCE: REGULATORY BIOL. LAB., SALK INST. BIOLOGICAL STUDIES, 10010 N. TORREY PINES RD., LA JOLLA, CALIF. 92037, USA.

SOURCE: NUCLEIC ACIDS RES, (1990) 18 (24), 7349-7356.

CODEN: NARHAD. ISSN: 0305-1048.

FILE SEGMENT: BA; OLD

LANGUAGE: English

AB We have generated a site-specific 17 bp insertion within a 38 kb chick globin gene cluster by employing the recombination abilities of *Saccharomyces cerevisiae*. This gene cluster contains four .beta.-type globin genes which share a high degree of sequence homology. In this procedure, a small fragment of .beta.A-globin DNA containing a 17 bp insertion is subcloned into a URA3-based yeast integrating vector (YIp). This mutated globin subclone is introduced into cells that carry the 38 kb globin cluster clone on a single-copy, \*\*\*circular\*\*\* \*\*\*vector\*\*\* derived from a yeast artificial chromosome (YAC). Insertion of the 17 bp oligomer is achieved by targeted integration of the YIp subclone. The recombinant contains the normal .beta.A-globin gene, the mutant gene and YIp vector sequences between the two copies. Excision of the vector sequences and one copy of the duplicated globin sequences by homologous recombination is required for cell survival when exposed to the selective agent 5-fluoroorotic acid, which is toxic to ura<sup>+</sup> yeast cells. Depending upon the point of the cross-over, a ura<sup>-</sup> yeast cell bearing either a wild-type globin gene or a 17 bp insertion mutation will result. By restriction mapping and in vitro transcription analysis, the .beta.A-globin gene containing the 17 bp insert has no nonspecific mutations generated during the recombination and selection procedures.

Specific mutations of regulatory regions, including protein-DNA binding sites, can be accurately targeted within extensive DNA clones by this method.

L2 ANSWER 31 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 16  
ACCESSION NUMBER: 1990:494131 BIOSIS  
DOCUMENT NUMBER: BA90:122477  
TITLE: EXPRESSION OF A FOREIGN KM-R GENE IN LINEAR KILLER DNA  
PLASMIDS IN YEAST.  
AUTHOR(S): TANGUY-ROUGEAU C; CHEN X J; WESOLOWSKI-LOUVEL M; FUKUHARAA  
H  
CORPORATE SOURCE: INST. CURIE, SECTION DE BIOLOGIE, BATIMENT 110, CENTRE  
UNIVERSITAIRE, 91405 ORSAY CEDEX, FRANCE.  
SOURCE: GENE (AMST), (1990) 91 (1), 43-50.  
CODEN: GENED6. ISSN: 0378-1119.  
FILE SEGMENT: BA; OLD  
LANGUAGE: English

AB The killer plasmids of the yeast *Kluyveromyces lactis*, pGKL1 and 2 (k1 and k2 for short), are linear double-stranded DNAs. The expression of genes of these plasmids is thought to depend on their own transcription system. Cloning the plasmid genes in conventional \*\*\*circular\*\*\*  
\*\*\*vectors\*\*\* is therefore not suitable for transcriptional studies, because such vectors use the host nuclear transcription system. In vitro modification of the linear plasmid genomes in order to introduce transcription reporter genes has been difficult because the structure of the plasmids, with covalently bound terminal proteins, does not allow their manipulation in vitro and amplification in *Escherichia coli*. We introduced the kanamycin/G418 resistance gene, KmR, into the k1 plasmid in vivo, by transforming the yeast with the linearized KmR gene bordered with short k1 sequences (part of the region encoding the toxin) to allow homologous recombination with the resident k1. In the linear recombinants obtained, however, the KmR was not expressed, while it was expressed if carried out circularized plasmids. By replacing the native promoter of KmR by the ORF1 promoter from k1, the KmR gene could be expressed in linear recombinants and conferred on the host a high level of resistance to the drug. All the linear recombinant plasmids were extremely stable under nonselective conditions. As a rare event, the integration of KmR produced a palindromic rearrangement of the k1 plasmid.

L2 ANSWER 32 OF 42 CAPLUS COPYRIGHT 2000 ACS  
ACCESSION NUMBER: 1990:527595 CAPLUS  
DOCUMENT NUMBER: 113:127595  
TITLE: Development of stable vectors for yeast based on  
linear killer plasmids of *Kluyveromyces lactis*  
AUTHOR(S): Kaemper, Joerg; Meinhardt, Friedhelm  
CORPORATE SOURCE: Ruhr-Univ., Bochum, D-4360, Fed. Rep. Ger.  
SOURCE: DECHEMA Biotechnol. Conf. (1989), 3(Pt. A, Jt. Meet.  
SIM DECHEMA, Presentation Biochem. Lab., Microb.  
Princ. Bioprocesses, Appl. Genet.), 351-5  
CODEN: DBCOEU  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The yeast *Kluyveromyces lactis* harbors 2 cytoplasmically localized linear DNA plasmids pGKL1 and GK11, responsible for the expression of a killer phenotype. Integration of the nuclear LEU2 gene into the smaller plasmid pGKL1 via in vivo recombination in *Saccharomyces cerevisiae* led to the attachment of telomeres to the ends. The plasmids are no longer

cytoplasmically localized, but nuclear assocd. Apparently, a nuclear gene cannot be expressed in the cytoplasm. In vivo recombination of pGKL1 with a LEU2 gene fused with pGKL1 promoter/terminator regions resulted in the formation of a cytoplasmically localized linear hybrid plasmid. This novel element corresponds to pGKL1 with respect to structure and organization. The newly developed vectors are extremely stable and have high copy nos. Thus, they presumably constitute an advantageous alternative to commonly used \*\*\*circular\*\*\* \*\*\*vectors\*\*\* .

L2 ANSWER 33 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 17  
ACCESSION NUMBER: 1989:223225 BIOSIS  
DOCUMENT NUMBER: BA87:114842  
TITLE: OLIGODEOXYNUCLEOTIDE-DIRECTED CLEAVAGE AND REPAIR OF A SINGLE-STRANDED VECTOR A METHOD OF SITE-SPECIFIC MUTAGENESIS.  
AUTHOR(S): AZHU D  
CORPORATE SOURCE: INST. JACQUES MONOD, CNRS UNIVERSITE PARIS 7, TOUR 43, 2 PLACE JUSSIEU, 75251 PARIS CEDEX 05, FRANCE.  
SOURCE: ANAL BIOCHEM, (1989) 177 (1), 120-124.  
CODEN: ANBCA2. ISSN: 0003-2697.  
FILE SEGMENT: BA; OLD  
LANGUAGE: English

AB A simple and efficient site-specific mutagenesis method is described. First, a single-stranded (ss) \*\*\*circular\*\*\* \*\*\*vector\*\*\* is linearized at the site where the desired mutation will be introduced. To do this, an oligodeoxynucleotide complementary to the target region of the ss vector and containing a restriction enzyme recognition sequence is annealed to the circular ss vector, and the partial double-strand formed is subsequently cleaved with that enzyme. Then, another oligodeoxynucleotide spanning the nick and carrying the mutation is annealed to the linearized ss DNA template and the annealed mixture is used directly to transform Escherichia coli without prior enzymatic DNA synthesis in vitro. The procedure has been applied successfully to constructing insertion, deletion, and point mutations in both M13 phage vectors and plasmid vectors containing the fl origin of replication.

L2 ANSWER 34 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 18  
ACCESSION NUMBER: 1988:352707 BIOSIS  
DOCUMENT NUMBER: BA86:48185  
TITLE: ENTRAPMENT OF RECOMBINANT PLASMIDS IN SEAPLAQUE AGAROSE PLUGS AND THEIR RAPID PURIFICATION FROM RECIRCULARIZED VECTORS.  
AUTHOR(S): UPCROFT P  
CORPORATE SOURCE: QUEENSLAND INST. MED. RES., BRAMSTON TERRACE, HERSTON, BRISBANE, QLD., AUSTRALIA 4006.  
SOURCE: GENE (AMST), (1988) 65 (2), 319-324.  
CODEN: GENED6. ISSN: 0378-1119.  
FILE SEGMENT: BA; OLD  
LANGUAGE: English

AB A simple method is described which permits both the separation and concentration of circular recombinant plasmids from smaller plasmid vectors that are an undesirable py-product of a ligation reaction. SeaPlaque agarose plugs are used to entrap open-circular forms of recombinant plasmids during electrophoresis. In the example described over 98% of supercoiled, open-circular and linear forms of the 2.9-kb Bluescript plasmid vector, as well as the equivalent dimer forms, pass through the 1.4% SeaPlaque plug. Circular recombinant plasmids greater in

length than the vector dimer are entrapped within the plug. By increasing the concentration of SeaPlaque, recombinants smaller than the vector dimer are retained in the trap, but with a concomitant increase in contamination by open- \*\*\*circular\*\*\* \*\*\*vector\*\*\* dimer. For most library constructions the high ratio of insert to vector used during the ligation reaction reduces the formation of vector dimers and makes this level of contamination inconsequential. The recombinant plasmids can be extracted readily from the SeaPlaque plug by excising it, melting the agarose and extracting with phenol. Alternatively, the excised plug can be melted and the recombinant plasmids used to transform bacteria, or mammalian cells, directly in the agarose. The procedure should be valuable for cloning large inserts for 'jumping' and 'linking' libraries, for large inserts in general where recircularisation is a low-frequency event, e.g., minichromosomes, for pulsed-field gel electrophoresis applications, and for hosts and vectors where genetic selection of the recombinant is not possible.

L2 ANSWER 35 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 19  
 ACCESSION NUMBER: 1988:352573 BIOSIS  
 DOCUMENT NUMBER: BA86:48051  
 TITLE: PLASMID PIJ699 A MULTI-COPY POSITIVE-SELECTION VECTOR FOR STREPTOMYCES.  
 AUTHOR(S): KIESER T; MELTON R E  
 CORPORATE SOURCE: JOHN INNES INST., COLNEY LANE, NORWICH NR4 7UH.  
 SOURCE: GENE (AMST), (1988) 65 (1), 83-92.  
 CODEN: GENED6. ISSN: 0378-1119.  
 FILE SEGMENT: BA; OLD  
 LANGUAGE: English

AB A plasmid vector, pIJ699, which provides positive selection for cloned DNA, was constructed using the replication functions of the Streptomyces wide-host-range multi-copy plasmid pIJ101. The selection for inserts is based on the principle that plasmids with long uninterrupted perfect palindromes (inverted repeats) are 'not viable' in bacteria. For cloning, pIJ699 is digested with BglII. This produces two fragments, one of which is the linearized vector, with two arms of the palindrome at its ends, and the other is a 'spacer' which is needed to keep the inverted repeat sequences apart. The vector fragment is separated from the 'spacer' fragment and ligated with the DNA to be cloned. Plasmids with a fragment of cloned DNA, but not the \*\*\*circularized\*\*\* \*\*\*vector\*\*\*, give rise to thiostrepton-resistant transformants in Streptomyces lividans. The inverted repeat sequences contain a strong transcription terminator which reduces transcriptional read-through both in and out of the cloned fragment. This improves the stability of many hybrid plasmids and facilitates the study of the regulation of cloned genes.

L2 ANSWER 36 OF 42 CAPLUS COPYRIGHT 2000 ACS  
 ACCESSION NUMBER: 1988:88960 CAPLUS  
 DOCUMENT NUMBER: 108:88960  
 TITLE: Cloning expression of hybrid polypeptides using recombinant vectors and in-vivo recombination  
 INVENTOR(S): Gray, Gregory L.  
 PATENT ASSIGNEE(S): Genencor, Inc., USA  
 SOURCE: Eur. Pat. Appl., 54 pp.  
 CODEN: EPXXDW  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 1

## PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 208491	A2	19870114	EP 1986-305057	19860630
EP 208491	A3	19880921		
EP 208491	B1	19930825		
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
AT 93541	E	19930915	AT 1986-305057	19860630
AU 8659450	A1	19870108	AU 1986-59450	19860701
CA 1312836	A1	19930119	CA 1986-512877	19860702
JP 62083891	A2	19870417	JP 1986-157163	19860703
JP 08029091	B4	19960327		
US 5093257	A	19920303	US 1989-431705	19891101
PRIORITY APPLN. INFO.:			US 1985-752267	19850703
			EP 1986-305057	19860630

AB \*\*\*Circular\*\*\* \*\*\*vectors\*\*\* contg. DNA sequences encoding all or part of .gtoreq.2 distinct parental polypeptides are used in processes utilizing in-vivo recombination to produce hybrid polypeptides. .alpha.-Amylase genes of Bacillus licheniformis and B. stearothermophilus are isolated, sequenced, cloned, and used to construct vectors for prodn. of hybrid amylases. The Escherichia coli-B. subtilis shuttle vector pBS42 contg. B. stearothermophilus-.alpha.-amylase gene lacking the C-terminal codons and the B. licheniformis-.alpha.-amylase gene lacking the N-terminal codons spaced by DNA sequence encoding the PstI restriction site was used to transform E. coli 294 (recA+). The amplified plasmids were isolated, treated with PstI, and used to retransform E. coli 294. Approx. 25% of the colonies isolated synthesized active hybrid .alpha.-amylase. The amino acid compn. and enzymic activity (compared to the parent polypeptides) of some of the hybrid proteins were analyzed.

L2 ANSWER 37 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 20  
 ACCESSION NUMBER: 1986:239210 BIOSIS  
 DOCUMENT NUMBER: BA82:3714  
 TITLE: THE SACCHAROMYCES-CEREVISIAE CHROMOSOME III LEFT TELOMERE HAS A TYPE X BUT NOT A TYPE Y' ARS REGION.  
 AUTHOR(S): BUTTON L L; ASTELL C R  
 CORPORATE SOURCE: DEP. BIOCHEM., FAC. MED., UNIV. B.C., VANCOUVER, B.C. V6T 1W5, CAN.  
 SOURCE: MOL CELL BIOL, (1986) 6 (4), 1352-1356.  
 CODEN: MCEBD4. ISSN: 0270-7306.  
 FILE SEGMENT: BA; OLD  
 LANGUAGE: English

AB A yeast Saccharomyces cerevisiae telomeric region was isolated by chromosome walking from HML.alpha., the most distal known gene on the chromosome III left (IIIL) end. The terminal heterodisperse 3,3-kilobase (kb) SalI fragments on chromosome IIIL, 8.6 kb distal to HML.alpha., was cloned in a \*\*\*circular\*\*\* \*\*\*vector\*\*\* to generate a telomeric probe. Southern hybridization and DNA sequencing analyses indicated that 0.6 kb (.+- .200 base pairs) of 5'-C1-3A-3' simple tandem repeat sequence, adjacent to a 1.2-kb type X ARS region, constitutes the telomere on the chromosome IIIL end, and no type Y' ARS region homologies exist between HML.alpha. and the IIIL terminus.

L2 ANSWER 38 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 21  
 ACCESSION NUMBER: 1987:125770 BIOSIS  
 DOCUMENT NUMBER: BA83:64831

TITLE: A LINEAR SHUTTLE VECTOR FOR YEAST AND THE HYPOTRICHIOUS  
CILIATE STYLONYCHIA.  
AUTHOR(S): ASCENZIONI F; LIPPS H J  
CORPORATE SOURCE: INST. BIOL. III, UNIV. TUEB., AUF DER MORGENSTELLE 28, 74  
TUEBINGEN, W. GER.  
SOURCE: GENE (AMST), (1986) 46 (1), 123-126.  
CODEN: GENED6. ISSN: 0378-1119.  
FILE SEGMENT: BA; OLD  
LANGUAGE: English

AB A linear plasmid was constructed in vitro using the telomeres of the rDNA  
of Tetrahymena pyriformis. These telomeres were added to a yeast  
\*\*\*circular\*\*\* \*\*\*vector\*\*\* containing an ARS sequence from  
Dictyostelium, the LEU2 gene of yeast and the neo gene from Escherichia  
coli Tn5 fused with a eukaryotic promoter. The resulting plasmid was used  
to transform yeast. During the replication of the linear plasmid in yeast  
it was spontaneously modified at the extremity by the addition of 300 bp  
of yeast telomeric sequence for each end. Total DNA prepared from yeast  
transformants was used to transform the hypotrichous ciliate Stylonychia  
lemnae. The same plasmid isolated from Stylonychia can again be replicated  
in yeast.

L2 ANSWER 39 OF 42 CAPLUS COPYRIGHT 2000 ACS  
ACCESSION NUMBER: 1985:417783 CAPLUS  
DOCUMENT NUMBER: 103:17783  
TITLE: Plasmid pGC18  
PATENT ASSIGNEE(S): Green Cross Corp., Japan  
SOURCE: Jpn. Kokai Tokkyo Koho, 14 pp.  
CODEN: JKXXAF  
DOCUMENT TYPE: Patent  
LANGUAGE: Japanese  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 60027388	A2	19850212	JP 1983-135032	19830722
EP 135045	A2	19850327	EP 1984-108599	19840720
EP 135045	A3	19870128		
EP 135045	B1	19901219		
R: BE, DE, FR, GB, NL, SE				
ES 534842	A1	19851201	ES 1984-534842	19840720
PRIORITY APPLN. INFO.:			JP 1983-135032	19830722

AB A \*\*\*circular\*\*\* \*\*\*vector\*\*\* plasmid, pGCu18 (5.7 kilobase pairs)  
which has a copy no. of 10-20 and contains 1 EcoRI and PvuII, 2 BamHI,  
HaeIII, and Bcl I, and 4 HindIII and HpaII restriction sites is isolated  
from Bacillus natto. Thus, B. natto GC18 was cultured in a medium contg.  
peptone, yeast ext., glucose, and NaCl overnight and centrifuged to  
collect the cultured cells. The cells were suspended in a TES soln. (pH  
8.0, 30 mM Tris-HCl, 50 mM NaCl, and 5 mM EDTA) contg. 25% sucrose, lysed  
with lysozyme and SDS, and centrifuged to recover a supernatant fraction.  
The supernatant fraction was pptd. with polyethylene glycol. The ppt. was  
redissolved and subjected to CsCl-ethidium bromide ultracentrifugation  
(60,000 rpm, 17 h) to obtain pCG18. The DNA sequences of the B. natto  
.alpha.-amylase gene promoter and terminator regions are also presented.

L2 ANSWER 40 OF 42 CAPLUS COPYRIGHT 2000 ACS  
ACCESSION NUMBER: 1985:18901 CAPLUS

DOCUMENT NUMBER: 102:18901  
 TITLE: Plasmid pOA7  
 PATENT ASSIGNEE(S): Sanraku-Ocean Co., Ltd., Japan  
 SOURCE: Jpn. Kokai Tokkyo Koho, 7 pp.  
 CODEN: JKXXAF  
 DOCUMENT TYPE: Patent  
 LANGUAGE: Japanese  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 59169493	A2	19840925	JP 1983-44463	19830318
AB	***circular***	***vector***	<p>Plasmid pOA7, a plasmid which transfers pock-forming activity to Streptomyces griseus ATCC 10137 is prep'd., pOA7 has a mol. wt. of .apprx.26.4 kilobase pairs and contains 1 EcoRI, 3 KpnI, 2 PstI, and &gt;9 SalI restriction endonuclease sites, but is not cleaved by BamHI, BglII, HindIII, and XbaI. Plasmid pOA7 was sep'd. from cultured Streptomyces species OA-7 (FERM p-6992) by lysozyme lysis and CsCl-ethidium bromide d. gradient centrifugation. The pOA7 prep'n. was used to transform S. griseus (ATCC 10137) by fusion of protoplasts from S. griseus with pOA and pock-forming transformants were selected; the transformant contained multicopies (.apprx.30 copies) of pOA7 and pOA7 was stable.</p>	

L2 ANSWER 41 OF 42 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1984:115833 CAPLUS  
 DOCUMENT NUMBER: 100:115833  
 TITLE: Creation of DNA sequences encoding modified proinsulin precursors  
 INVENTOR(S): Seed, Brian S.  
 PATENT ASSIGNEE(S): Genetics Institute, USA  
 SOURCE: Eur. Pat. Appl., 40 pp.  
 CODEN: EPXXDW  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 90433	A1	19831005	EP 1983-103253	19830331
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
WO 8303413	A1	19831013	WO 1983-US482	19830331
W: AU, DK, FI, HU, KP, MC, NO				
AU 8315594	A1	19831024	AU 1983-15594	19830331
JP 58183659	A2	19831026	JP 1983-56798	19830331
HU 32837	O	19840928	HU 1983-2264	19830331
FI 8304213	A	19831117	FI 1983-4213	19831117
DK 8305360	A	19831123	DK 1983-5360	19831123
NO 8304392	A	19831129	NO 1983-4392	19831129
PRIORITY APPLN. INFO.:			US 1982-363657	19820331
			WO 1983-US482	19830331

AB A modified human proinsulin, which may be processed to insulin [9004-10-8] in vitro is constructed by substituting aspartate at the N terminus (position 31 of proinsulin) and methionine at the C terminus (position 65 of proinsulin) of the C chain by annealing a primer

oligonucleotide contg. the appropriate triplet to single-stranded DNA of vector M13, contg. human proinsulin cDNA, and cloning in Escherichia coli. The proinsulin which has no aspartate or methionine residues in the A or B chain is processed in vitro with the use of aspartate-specific P. fragi mutant Mel enzyme [55576-49-3] and methionine-specific trypsin [9002-07-7]. The doubly-substituted proinsulin cDNA-contg. vectors were cloned in E. coli and double-stranded cDNA isolated and fused to a 2nd vector known to express high levels of proinsulin in prodn. cells. Thus, the primer d(CTCCCGATCGGTCTT) [88384-67-2] contg. the aspartate-encoding triplet ATC and the restriction endonuclease PvuI [81295-33-2] recognition sequence CGATCG, as well as sequences complementary to the message-synonomous cDNA encoding amino acids at positions 29, 30, 32, and 33 of proinsulin, was annealed to single-stranded M13 contg. human proinsulin cDNA. After annealing, the sequence was extended with DNA polymerase and the double-stranded \*\*\*circular\*\*\* \*\*\*vector\*\*\* was cloned in E. coli. Microbiol clones contg. modified proinsulin DNA were identified with the use of PvuI and electrophoresis of digestion products, cultured in liq. media, and the single-stranded vectors extruded from the cells and contg. modified cDNA were recovered. Methionine was substituted, similarly, at the N terminus (position 65) of the C chain with the use of the recovered cDNA and primer AATCCCCATCTTCTG [88384-68-3] contg. the restriction endonuclease MboII [81295-29-6] recognition sequence and sequences complementary to the message-synonomous cDNA encoding amino acids at positions 63, 64, 66, and 67 of proinsulin. The modified proinsulin was recovered after adhering to columns contg. monoclonal antibodies to C chain sequences, and enzymically processed in vitro.

L2 ANSWER 42 OF 42 CAPLUS COPYRIGHT 2000 ACS  
 ACCESSION NUMBER: 1975:60688 CAPLUS  
 DOCUMENT NUMBER: 82:60688  
 TITLE: Gas desorption apparatus  
 INVENTOR(S): Miller, Donald Stuart  
 PATENT ASSIGNEE(S): British Hydromechanics Research Assoc.  
 SOURCE: Ger. Offen., 22 pp.  
 CODEN: GWXXBX  
 DOCUMENT TYPE: Patent  
 LANGUAGE: German  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 2419676	A1	19741031	DE 1974-2419676	19740424
DE 2419676	C2	19890601		
GB 1461591	A	19770113	GB 1973-19214	19740411
NL 7405510	A	19741028	NL 1974-5510	19740424
NL 185895	B	19900316		
NL 185895	C	19900816		
JP 50030175	A2	19750326	JP 1974-46421	19740424
JP 59008403	B4	19840224		

PRIORITY APPLN. INFO.: GB 1973-19214 19730424

AB The liq. contg. the gas to be removed is injected through a nozzle into the top of a desorption column, which can be a simple cylinder or a series of cylinders of progressively decreasing diams. like an extended telescope. In either case, the column is long enough so that the jet fills the entire diam. of the bottom of the column, thus sealing a space

between the jet and the column wall near the nozzle from the space above the liq. level in the vacuum vessel below the column. This space in the column ensures that the gas is released in the jet due to the drop in pressure in the space. Thus, when the jet enters the vacuum tank below the column, the gas bubbles to the surface and is removed by a vacuum pump. The liq. is removed in amts. controlled by a level detector. The vessel can be fitted with a deflector just under the end of the column, and the nozzle can be fitted with either a butterfly valve to impart a \*\*\*circular\*\*\* \*\*\*vector\*\*\* to the jet or an adjustable centerpiece such as that in a garden-hose nozzle.

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(FILE 'HOME' ENTERED AT 11:17:55 ON 02 MAR 2000)

FILE 'MEDLINE, BIOSIS, SCISEARCH, EMBASE, CAPLUS' ENTERED AT 11:18:08 ON  
02 MAR 2000

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L1      1297 S VECTOR AND LINEAR? AND CIRCULAR?
L2      11 S L1 AND (PEG OR POLYETHYLENE()) GLYCOL)
L3      4 DUP REM L2 (7 DUPLICATES REMOVED)
L4      5 S L1 AND TOPOISOMERASE
L5      1 DUP REM L4 (4 DUPLICATES REMOVED)

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=> d 13 ibib abs 1-4

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L5 ANSWER 1 OF 1 MEDLINE
ACCESSION NUMBER: 97152589 MEDLINE
DOCUMENT NUMBER: 97152589
TITLE: DNA ***topoisomerase*** II inhibitors enhance random
integration of transfected ***vectors*** into human
chromosomes.
AUTHOR: Fujimaki K; Aratani Y; Fujisawa S; Motomura S; Okubo T;
Koyama H
CORPORATE SOURCE: Kihara Institute for Biological Research, Yokohama City
University, Japan.
SOURCE: SOMATIC CELL AND MOLECULAR GENETICS, (1996 Jul) 22 (4)
279-90.
JOURNAL CODE: UY2. ISSN: 0740-7750.
PUB. COUNTRY: United States
JOURNAL; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199704
ENTRY WEEK: 19970403
AB To study the involvement of DNA ***topoisomerase*** (topo) II on
nonhomologous (illegitimate) recombination, we examined the effect of topo
II inhibitors on random integration of exogenous ***vectors*** into
human chromosomes. We transfected human cell lines PA1, HeLa and EJ-1 with
***linearized*** plasmid pSV2neo by electroporation, treated with topo
II inhibitors and determined the frequency of Geneticin-resistant (G418r)
colonies. We found that three topo II inhibitors, etoposide (VP-16),
ICRF-193 and amsacrine (m-AMSA), greatly enhanced the frequency of G418r
colonies. These effects were maximally expressed by as little as 12 hrs
treatment with the drugs. Similar enhancements were found with different

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\*\*\*vectors\*\*\* (closed- \*\*\*circular\*\*\* and \*\*\*linear\*\*\* ), different cell types, or by different transfection methods (calcium precipitation and lipofection). In contrast, the inhibitor treatments did not affect the transient expression of chloramphenicol acetyltransferase and beta-galactosidase activity following transfection with pSV2CAT and pCH110, respectively. Southern blot analysis revealed that the integration pattern of transfected pSV2neo into PA1 chromosomes was random and not characteristic for each inhibitor. These results suggest that topo II inhibitors directly act at a nonhomologous recombination reaction, promoting the integration process of transfected \*\*\*vectors\*\*\* into human chromosomes. We discuss the enhancement mechanism with a special emphasis on DNA strand breaks induced by the inhibitors.

L3 ANSWER 1 OF 4 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 1  
 ACCESSION NUMBER: 1994:204717 BIOSIS  
 DOCUMENT NUMBER: PREV199497217717  
 TITLE: Screening for Sulfolobales, their plasmids and their viruses in Icelandic solfataras.  
 AUTHOR(S): Zillig, Wolfram (1); Kletzin, Arnulf; Schleper, Christa; Holz, Ingelore; Janekovic, Davorin; Hain, Johannes; Lanzendoerfer, Martin; Kristjansson, Jakob K.  
 CORPORATE SOURCE: (1) Max Planck-Inst. Biochemie, Martinsried Germany  
 SOURCE: Systematic and Applied Microbiology, (1994) Vol. 16, No. 4, pp. 609-628.  
 ISSN: 0723-2020.  
 DOCUMENT TYPE: Article  
 LANGUAGE: English  
 AB We have sampled acidic springs, water and mud holes of 14 major solfataric fields of Iceland in order to isolate both heterotrophic and autotrophic members of the order Sulfolobales and to find affiliated genetic elements e.g. plasmids and viruses. The diversity of 120 isolates was analysed by comparing DNA restriction fragment patterns. The 44 heterotrophic isolates belonged to only two types, the solfataricus (S) type and the islandicus (I) type as judged by the restriction patterns of their DNAs. None of the heterotrophic isolates was able to grow autotrophically. The more than 70 autotrophic isolates belonged to only three types, the Desulfurolobus (D) type, the closely related A type and, rarely, the B type. None of the autotrophic isolates was able to grow heterotrophically. Primary heterotrophic colonies often gave rise to obligately autotrophic isolates when submitted to autotrophic selection, probably because they constituted syntrophic associations. Inversely, primary autotrophic colonies sometimes yielded obligately heterotrophic isolates when submitted to heterotrophic selection. Cell-free filtrates of the field samples precipitated with \*\*\*PEG\*\*\* 6000 yielded different types of virus-like particles as visualized by electron microscopy. Some of these were probably Thermoproteus viruses. No infectious or even lytic virus was obtained from these samples. Four different multicopy plasmids, three, pRN1, pRN2 and pHE7 from heterotrophic hosts and one, pDL10, originally found in Desulfurolobus ambivalens DSM 3772, occurring in all but two autotrophs of the D type, were characterized and used for developing cloning \*\*\*vectors\*\*\*. All 18 representatives of the heterotrophic S type and none of the I type were lysogenic for the virus SSV1 originally discovered in S. shibatae. Different lysogens exhibited different ratios of free \*\*\*circular\*\*\* and \*\*\*linearly\*\*\* integrated viral DNA. The I type isolate KVEM10H1 multiplied SSV 1 but did not integrate its

genome into the chromosome. One heterotrophic I type and one autotrophic D type isolate carried double-stranded DNA viruses, SIRV and DAFV. SIRV is a stiff rod, 0.95  $\mu\text{m}$  long and 0.026  $\mu\text{m}$  in diameter, consisting of 33 kb pairs of \*\*\*linear\*\*\* double stranded DNA, a strongly basic DNA binding protein and terminal tentacles involved in attachment to thin filaments, most probably pili. It lacks a membrane or hydrophobic coat and represents a novel virus type. The flexible filamentous DAFV, 2.3  $\mu\text{m}$  long and 0.027  $\mu\text{m}$  in diameter, containing 56 kb pairs of \*\*\*linear\*\*\* double-stranded DNA, appears to be enwrapped in a membrane. It resembles representatives of the lipotrixviridae, most closely the virus TTV2.

L3 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1990:193148 CAPLUS  
 DOCUMENT NUMBER: 112:193148  
 TITLE: Introduction of a yeast artificial chromosome  
 \*\*\*vector\*\*\* into *Saccharomyces cerevisiae* cells by  
 electroporation  
 AUTHOR(S): Rech, E. L.; Dobson, M. J.; Davey, M. R.; Mulligan, B.  
 J.  
 CORPORATE SOURCE: Dep. Bot., Univ. Nottingham, Nottingham, NG7 2RD, UK  
 SOURCE: Nucleic Acids Res. (1990), 18(5), 1313  
 CODEN: NARHAD; ISSN: 0305-1048  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English

AB A yeast artificial chromosome \*\*\*vector\*\*\* was introduced into intact yeast cells by electroporation, both as a closed \*\*\*circular\*\*\* plasmid and as a \*\*\*linear\*\*\* mol. with terminal telomeres. The procedure employed lower field strengths, higher capacitance and lower resistance than used previously to minimize heat dissipation. The \*\*\*vector\*\*\*, pYAC4 with a short oligonucleotide inserted at the EcoRI cloning site to disrupt the SUP4 reading frame, was used to transform *S. cerevisiae* AB 1380 to a Ura<sup>+</sup> Trp<sup>+</sup> red phenotype. The frequency of transformation was dependent on the electroporation parameters and was a function of DNA concn., cell no., and \*\*\*PEG\*\*\* concn. \*\*\*Linearization\*\*\* of the plasmid increased the no. of transformants about 4-fold compared to \*\*\*circular\*\*\* plasmid. Approx. 99% of the colonies had the expected Ura<sup>+</sup> Trp<sup>+</sup> red phenotype. This protocol forms the basis for examn. of electroporation as a means for direct delivery of large YAC clones into yeast without the need for a spheroplast stage.

L3 ANSWER 3 OF 4 MEDLINE

DUPLICATE 2

ACCESSION NUMBER: 88124941 MEDLINE  
 DOCUMENT NUMBER: 88124941  
 TITLE: Gene transfer system for the phytopathogenic fungus  
 Ustilago maydis.  
 AUTHOR: Wang J; Holden D W; Leong S A  
 CORPORATE SOURCE: U.S. Department of Agriculture-Agricultural Research  
 Service, Madison, WI.  
 CONTRACT NUMBER: 1 R01 GM33716 (NIGMS)  
 SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE  
 UNITED STATES OF AMERICA, (1988 Feb) 85 (3) 865-9.  
 Journal code: PV3. ISSN: 0027-8424.  
 PUB. COUNTRY: United States  
 Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals; Cancer Journals  
 ENTRY MONTH: 198805

AB A selectable marker for transformation was constructed by transcriptional fusion of a *Ustilago maydis* heat shock gene promoter with the hygromycin B phosphotransferase gene of *Escherichia coli*. *U. maydis* was transformed to hygromycin B resistance by \*\*\*polyethylene\*\*\* \*\*\*glycol\*\*\* -induced fusion of spheroplasts following exposure to plasmid DNA that carried the marker gene. Transformation frequencies of 50 and 1000 transformants per microgram of DNA per  $2 \times 10^7$  spheroplasts were obtained for \*\*\*circular\*\*\* and \*\*\*linear\*\*\* \*\*\*vector\*\*\* DNA, respectively. In the majority of transformants, the \*\*\*vector\*\*\* was integrated at a single chromosomal site, in either single copy or tandem duplication, as determined by Southern hybridization analysis of electrophoretically separated chromosomes and of restriction-endonuclease-cleaved DNA. The predominant form (82%) of \*\*\*vector\*\*\* integration was by nonhomologous recombination; the remainder carried the plasmid at the homologous heat shock gene locus. No evidence for gene conversion or gene replacement was obtained in 28 transformants. Hygromycin B phosphotransferase activity and resistance to hygromycin B were roughly correlated with the copy number of the integrated \*\*\*vector\*\*\* at the homologous location. Transforming DNA was stably maintained during mitosis and meiosis. This transformation procedure and associated \*\*\*vector\*\*\* should permit the cloning of genes by direct complementation in *U. maydis*.

L3 ANSWER 4 OF 4 MEDLINE DUPLICATE 3  
 ACCESSION NUMBER: 85021327 MEDLINE  
 DOCUMENT NUMBER: 85021327  
 TITLE: Plasmid transformation of *Streptococcus lactis* protoplasts: optimization and use in molecular cloning.  
 AUTHOR: Kondo J K; McKay L L  
 SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1984 Aug) 48 (2) 252-9.  
 Journal code: 6K6. ISSN: 0099-2240.  
 PUB. COUNTRY: United States  
 Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 198501

AB The parameters affecting \*\*\*polyethylene\*\*\* \*\*\*glycol\*\*\* -induced plasmid transformation of *Streptococcus lactis* LM0230 protoplasts were examined to increase the transformation frequency. In contrast to spreading protoplasts over the surface of an agar medium, their incorporation into soft agar overlays enhanced regeneration of protoplasts and eliminated variability in transformation frequencies. \*\*\*Polyethylene\*\*\* \*\*\*glycol\*\*\* with a molecular weight of 3,350 at a final concentration of 22.5% yielded optimal transformation. A 20-min \*\*\*polyethylene\*\*\* \*\*\*glycol\*\*\* treatment of protoplasts in the presence of DNA was necessary for maximal transformation. The number of transformants recovered increased as the protoplast and DNA concentration increased over a range of  $3.0 \times 10^6$  to  $3.0 \times 10^8$  protoplasts and 0.25 to 4.0 micrograms of DNA per assay, respectively. With these parameters, transformation was increased to  $5 \times 10^3$  to  $4 \times 10^4$  transformants per microgram of DNA. \*\*\*Linear\*\*\* and recombinant plasmid DNA transformed, but at frequencies 10- to 100-fold lower than that of covalently closed \*\*\*circular\*\*\* DNA. Transformation of recombinant DNA molecules enabled the cloning of restriction endonuclease fragments coding for lactose metabolism into *S. lactis* LM0230 with the *Streptococcus sanguis* cloning \*\*\*vector\*\*\*, pGB301. These results demonstrated that the transformation frequency is sufficient to clone plasmid-coded genes

which should prove useful for strain improvement of dairy starter cultures.

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L1 206 (VECTOR OR PLASMID) AND TERMINASE  
L2 0 L1 AND (POLYETHYLENE(W) GLYCOL OR PEG)  
L3 18 L1 AND (TRANSFORM? OR TRANSFECT?)  
L4 10 DUP REM L3 (8 DUPLICATES REMOVED)

L4 ANSWER 1 OF 10 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 1998281527 EMBASE

TITLE: Bacteriophage P2 and P4 morphogenesis: Structure and function of the connector.

AUTHOR: Rishovd S.; Holzenburg A.; Johansen B.V.; Lindqvist B.H.

CORPORATE SOURCE: B.H. Lindqvist, Inst. of Biol./Biotechnol. Ctr. Oslo, University of Oslo, P.O. Box 1125, Blindern N-0317 Oslo, Norway

SOURCE: Virology, (25 May 1998) 245/1 (11-17).

Refs: 40

ISSN: 0042-6822 CODEN: VIRLAX

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The connector, the structure located between the bacteriophage capsid and tail, is interesting from several points of view. The connector is in many cases involved in the initiation of the capsid assembly process, functions as a gate for DNA transport in and out of the capsid, and is, as implied by the name, the structure connecting a tail to the capsid. Occupying a position on a 5-fold axis in the capsid and connected to a coaxial 6-fold tail, it mediates a symmetry mismatch between the two. To understand how the connector is capable of all these interactions its structure needs to be worked out. We have focused on the bacteriophage P2/P4 connector, and here we report an image reconstruction based on 2D crystalline layers of connector protein expressed from a \*\*\*plasmid\*\*\* in the absence of other phage proteins. The overall design of the connector complies well with that of other phage connectors, being a toroid structure having a conspicuous central channel. Our data suggests a 12-fold symmetry, i.e., 12 protrusions emerge from the more compact central part of the structure. However, rotational analysis of single particles suggests that there are both 12- and 13-mers present in the crude sample. The connectors used in this image reconstruction work differ from connectors in virions by having retained the amine-terminal 26 amine acids normally cleaved off during the morphogenetic process. We have used different late gene mutants to demonstrate that this processing occurs during DNA packaging, since only mutants in gene P, coding for the large \*\*\*terminase\*\*\* subunit, accumulate uncleaved connector protein. The suggestion that the cleavage might be intimately involved in the DNA packaging process is substantiated by the fact that the fragment cleaved off is highly basic and is homologous to known DNA binding sequences.

L4 ANSWER 2 OF 10 MEDLINE  
 ACCESSION NUMBER: 97181626 MEDLINE  
 DOCUMENT NUMBER: 97181626  
 TITLE: Mutational analysis of bacteriophage phi CTX cos site.  
 AUTHOR: Xiong G; Lutz F  
 CORPORATE SOURCE: Institut fur Pharmakologie und Toxikologie, Universitat  
 Giessen, Germany.  
 SOURCE: VIRUS RESEARCH, (1996 Dec) 46 (1-2) 149-56.  
 Journal code: X98. ISSN: 0168-1702.  
 PUB. COUNTRY: Netherlands  
 Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199706  
 ENTRY WEEK: 19970603

AB The DNA of phi CTX contains the gene ctx, located closely downstream of  
 cos. This encodes for the pore-forming cytotoxin protein, CTX. phi CTX  
 converts some Pseudomonas aeruginosa strains into CTX producers. After  
 different periods of phi CTX infection, two distinct forms of phage DNA  
 were isolated: circular DNA from bacterial cytosol and later linear DNA  
 from phi CTX particles. When circular phi CTX DNA was \*\*\*transfected\*\*\*  
 into the P. aeruginosa strains CF5 and E40, phi CTX was amplified and ctx  
 expressed. phi CTX induced a protein fraction in CF5 cells that cleaved  
 the 0.477 kb cos fragment at the cos site, indicating \*\*\*terminase\*\*\*  
 activity. Deletion and point mutation variants of the cos DNA were  
 prepared. Protein binding to DNA in vitro and competition experiments in  
 vivo showed that portions of the cos site and its flanking sequences are  
 differentially critical to the binding of phi CTX-induced proteins.

L4 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2000 ACS  
 ACCESSION NUMBER: 1991:557158 CAPLUS  
 DOCUMENT NUMBER: 115:157158  
 TITLE: Recombinant manufacture of .lambda.- \*\*\*terminase\*\*\*  
 INVENTOR(S): Nakatsuji, Masako; Kotani, Hiroichi; Ito, Hiroyuki;  
 Takahashi, Asako; Hiraoka, Shinji; Nakamura, Teruya  
 PATENT ASSIGNEE(S): Takara Shuzo Co., Ltd., Japan  
 SOURCE: Jpn. Kokai Tokkyo Koho, 6 pp.  
 CODEN: JKXXAF  
 DOCUMENT TYPE: Patent  
 LANGUAGE: Japanese  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 03143392	A2	19910618	JP 1989-279671	19891030
JP 07112429	B4	19951206		

AB .lambda.- \*\*\*Terminase\*\*\*, lacking contamination by nuclease which  
 inhibits the cleavage at the COS termini, is manufd. by co-expression of  
 the Nul and A genes. \*\*\*Plasmid\*\*\* pKTNul and pKTA-1 for expression  
 in Escherichia coli of the Nul gene and the A gene, resp., were prepd.  
 Cleavage of .lambda.-DNA inserted in the rho site or the att .lambda. site  
 of E. coli chromosomal DNA using the .lambda.- \*\*\*terminase\*\*\* purified  
 from the \*\*\*transformants\*\*\* was also demonstrated.

L4 ANSWER 4 OF 10 MEDLINE  
 ACCESSION NUMBER: 91317727 MEDLINE  
 DUPLICATE 1

DOCUMENT NUMBER: 91317727  
TITLE: Isolation and characterization of mutations in the bacteriophage lambda \*\*\*terminase\*\*\* genes.  
AUTHOR: Davidson A; Yau P; Murialdo H; Gold M  
CORPORATE SOURCE: Department of Molecular and Medical Genetics, University of Toronto, Canada..  
SOURCE: JOURNAL OF BACTERIOLOGY, (1991 Aug) 173 (16) 5086-96.  
Journal code: HH3. ISSN: 0021-9193.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199111

AB The \*\*\*terminase\*\*\* enzyme of bacteriophage lambda is a hetero-oligomeric protein which catalyzes the site-specific endonucleolytic cleavage of lambda DNA and its packaging into phage proheads; it is composed of the products of the lambda Nul and A genes. We have developed a simple method to select mutations in the \*\*\*terminase\*\*\* genes carried on a high-copy-number \*\*\*plasmid\*\*\*, based on the ability of wild-type \*\*\*terminase\*\*\* to kill recA strains of Escherichia coli. Sixty-three different spontaneous mutations and 13 linker insertion mutations were isolated by this method and analyzed. Extracts of cells \*\*\*transformed\*\*\* by mutant \*\*\*plasmids\*\*\* displayed variable degrees of reduction in the activity of one or both \*\*\*terminase\*\*\* subunits as assayed by in vitro lambda DNA packaging. A method of genetically mapping \*\*\*plasmid\*\*\* -borne mutations in the A gene by measuring their ability to rescue various lambda Aam phages showed that the A mutations were fairly evenly distributed across the gene. Mutant A genes were also subcloned into overproducing \*\*\*plasmid\*\*\* constructs, and it was determined that more than half of them directed the synthesis of normal amounts of full-length A protein. Three of the A gene mutants displayed dramatically reduced in vitro packaging activity only when immature (uncut) lambda DNA was used as the substrate; therefore, these mutations may lie in the endonuclease domain of \*\*\*terminase\*\*\*. Interestingly, the putative endonuclease mutations mapped in two distinct locations in the A gene separated by a least 400 bp.

L4 ANSWER 5 OF 10 MEDLINE

ACCESSION NUMBER: 91311675 MEDLINE  
DOCUMENT NUMBER: 91311675  
TITLE: The last duplex base-pair of the phage lambda chromosome. Involvement in packaging, ejection and routing of lambda DNA.  
AUTHOR: Xu S Y; Feiss M  
CORPORATE SOURCE: Department of Microbiology, University of Iowa, Iowa City 52242..  
CONTRACT NUMBER: AI12581 (NIAID)  
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1991 Jul 20) 220 (2) 293-306.  
Journal code: J6V. ISSN: 0022-2836.  
PUB. COUNTRY: ENGLAND: United Kingdom  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; Cancer Journals  
ENTRY MONTH: 199110

AB cosN is the site at which the bacteriophage lambda DNA packaging enzyme, \*\*\*terminase\*\*\*, introduces staggered nicks to generate the cohesive

ends of mature lambda chromosomes. Genetic and molecular studies show that cosN is recognized specifically by \*\*\*terminase\*\*\* and that effects of cosN mutations on lambda DNA packaging and cosN cleavage are well correlated. Mutations affecting a particular base-pair of cosN are unusual in being lethal in spite of causing only a moderate defect in cosN cleavage and DNA packaging. The particular base-pair is the rightmost duplex base-pair in mature chromosomes, at position 48,502 in the numbering system of Daniels et al; herein called position - 1. A G.C to T.A transversion mutation at position - 1, called cosN - 1T, reduces the particle yield of lambda fivefold, and the particles formed are not infectious. lambda cosN - 1T particles have wild-type morphology, and contain chromosomes that have normal cohesive ends. The chromosomes of lambda cosN - 1T particles, like the chromosomes of lambda + particles, are associated with the tail. lambda cosN - 1T particles, in spite of being normal structurally, are defective in injection of DNA into a host cell. Only approximately 25% of lambda cosN - 1T particles are able to eject DNA from the capsid in contrast to 100% for lambda +. Furthermore, for the 25% that do eject, there is a further injection defect because the ejected lambda cosN - 1T chromosomes fail to cyclize, in contrast to the efficient cyclization found for wild-type chromosomes following injection. The cosN - 1T mutation has no effect on Ca<sup>2+</sup> mediated

\*\*\*transformation\*\*\* by lambda DNA, indicating that the effect of the mutation on DNA fate is specific to the process of DNA injection. Models in which specific DNA : protein interactions necessary for DNA injection, and involving the rightmost base-pair of the lambda chromosome, are considered.

L4 ANSWER 6 OF 10 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 2  
 ACCESSION NUMBER: 1991:455046 BIOSIS  
 DOCUMENT NUMBER: BA92:99826  
 TITLE: CONSTRUCTION OF COSMID LIBRARY AND DETAILED PHYSICAL MAP OF RICE CHLOROPLAST.  
 AUTHOR(S): ZHAO Y; WENG X; ZOU Q; SHEN G; TANG S; CHAI J; WANG X  
 CORPORATE SOURCE: DEP. BIOL., HANGZHOU UNIV., HANGZHOU 310029.  
 SOURCE: ACTA GENET SIN, (1991) 18 (2), 149-160.  
 CODEN: ICHPCG. ISSN: 0379-4172.  
 FILE SEGMENT: BA; OLD  
 LANGUAGE: Chinese

AB The intact rice chloroplast was isolated by homogenizing rice leaves in a buffer containing ascorbic acid of high concentration and centrifuging. The obtained rice chloroplast DNA (ctDNA) is in high yield (100 .mu.g/100g leaves) and pure enough for restriction endonuclease analysis. The ctDNA fragments generated by partial digestion with suitable restriction endonucleases were inserted into the \*\*\*vector\*\*\* - pcos2 EMBL, and the recombinant DNAs were packaged in vitro and \*\*\*transfected\*\*\* the host bacteria cells. The tetracycline-sensitive and kanamycine-resistant recombinants were screened and the cloning efficiency approached over 10,000/.mu.g inserted DNA. The recombinant DNAs were linearized, digested by lambda- \*\*\*terminase\*\*\* as cos site, partially digested by restriction endonucleases and hybridized with [.gamma.-32P] ATP-labeled oligonucleotide lambda mapping probes. Then the recombinant DNAs were separated by electrophoresis and a detailed restriction endonuclease map has been constructed from the autoradiograms. The rice ctDNA has a length of 129.5 kb and 11, 12, 17, 37, 67 and 44 recognition sites of Puv II, Sal I, Pst I, Hind II, EcoR I and BamH I, respectively. The inserted repeat(IR) sequence has a length of 21.7 kb, the large single copy(LSC) is 73.7 kb and small single copy (SSC), 12.4 kb.

L4 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1990:17372 CAPLUS  
DOCUMENT NUMBER: 112:17372  
TITLE: Transphamids - novel bacteriophage .lambda./  
\*\*\*plasmid\*\*\* fusions as cloning \*\*\*vectors\*\*\*  
INVENTOR(S): Kiss, Gyorgy Botond; Vincze, Eva; Ott, Istvan; Kiss,  
Peter; Klupp, Tibor; Molnar, Istvan; Szeleczy,  
Zoltan; Ambrus, Gabor; Moravcsik, Imre  
PATENT ASSIGNEE(S): Biogal Gyogyszergyar, Hung.  
SOURCE: Eur. Pat. Appl., 44 pp.  
CODEN: EPXXDW  
DOCUMENT TYPE: Patent  
LANGUAGE: German  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 308883	A2	19890329	EP 1988-115460	19880921
EP 308883	A3	19900704		
EP 308883	B1	19950517		
R: AT, BE, CH, DE, ES, FR, GB, IT, LI, NL, SE				
HU 47634	A2	19890328	HU 1987-4245	19870921
HU 204892	B	19920228		
FI 8804346	A	19890322	FI 1988-4346	19880921
JP 01157392	A2	19890620	JP 1988-237474	19880921
ES 2075833	T3	19951016	ES 1988-115460	19880921
PRIORITY APPLN. INFO.:			HU 1987-4245	19870921

AB Hybrid mols. (transphamids) contg. the genes for the essential functions of .lambda. EMBL4 and a \*\*\*plasmid\*\*\* origin of replication are described. These \*\*\*vectors\*\*\* are 32 kb long and can accept up to 18 kb of foreign DNA, they replicate in host cells as \*\*\*plasmids\*\*\* and can be recovered as such. The recombinant mols. can be packaged in vitro after a ligation mix contg. concatameric DNA is reduced to monomers with .lambda. \*\*\*terminase\*\*\*. The size of the \*\*\*vector\*\*\* is such that only transphamids contg. an insert will be packaged in vitro. Antibiotic resistance encoded by the \*\*\*plasmid\*\*\* sequences can be used as a selective marker. The manuf. of gene banks with these \*\*\*vectors\*\*\* was approx. 10-fold more efficient than with present phage and \*\*\*plasmid\*\*\*. \*\*\*vectors\*\*\*. Novel phasmid \*\*\*vectors\*\*\* were also described. A gene bank from DNA of Streptomyces tenebrarius prepd. with a transphamid \*\*\*vector\*\*\* showed all antibiotic-resistant \*\*\*transformants\*\*\* contg. inserts.

L4 ANSWER 8 OF 10 MEDLINE

DUPLICATE 3

ACCESSION NUMBER: 88072068 MEDLINE  
DOCUMENT NUMBER: 88072068  
TITLE: A novel in vitro DNA packaging system demonstrating a direct role for the bacteriophage lambda FI gene product.  
AUTHOR: Davidson A; Gold M  
CORPORATE SOURCE: Department of Medical Genetics, University of Toronto, Ontario, Canada.  
SOURCE: VIROLOGY, (1987 Dec) 161 (2) 305-14.  
Journal code: XEA. ISSN: 0042-6822.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English  
FILE SEGMENT: Priority Journals; Cancer Journals  
ENTRY MONTH: 198803

AB A new in vitro bacteriophage lambda DNA packaging system is described in which all the proteins necessary for head morphogenesis are supplied by extracts of \*\*\*plasmid\*\*\* - \*\*\*transformed\*\*\* cells. This assay is used to demonstrate that the lambda FI gene product (gpFI) is necessary for maximal packaging efficiency when proheads and \*\*\*terminase\*\*\* are present in limiting amounts. A 100- to 200-fold decrease in packaging is seen when gpFI is omitted. gpFI is shown to act at and/or after the stage in packaging where proheads bind to the DNA: \*\*\*terminase\*\*\* complex.

L4 ANSWER 9 OF 10 BIOSIS COPYRIGHT 2000 BIOSIS  
ACCESSION NUMBER: 1988:155131 BIOSIS  
DOCUMENT NUMBER: BA85:78784  
TITLE: THE OVERPRODUCTION OF DNA \*\*\*TERMINASE\*\*\* OF COLIPHAGE LAMBDA.  
AUTHOR(S): CHOW S; DAUB E; MURIALDO H  
CORPORATE SOURCE: DEP. MED. GENETICS, MED. SCI. BUILD., UNIV. TORONTO, TORONTO, ONTARIO M5S 1A8, CAN.  
SOURCE: GENE (AMST), (1987) 60 (2-3), 277-290.  
CODEN: GENED6. ISSN: 0378-1119.  
FILE SEGMENT: BA; OLD  
LANGUAGE: English

AB An artificial operon containing the genes coding for the two subunits of .lambda. DNA \*\*\*terminase\*\*\*, Nul and A, has been constructed. Derivatives of \*\*\*plasmid\*\*\* pBR322 served as the cloning vehicles. The transcription is driven by the pL promoter of phage .lambda., and translation of the \*\*\*terminase\*\*\* genes was made efficient by the replacement of the wild-type ribosome-binding sites for those of .lambda. genes cII and/or D. The operon also carries the oL operator, and this enables regulation of its expression by a thermosensitive repressor. The synthesis of genes Nul and A products is extremely efficient upon derepression. Within 40 min after induction of the operon, the two subunits comprise about 20% of the total cellular protein mass. Crude extracts prepared from these overproducing strains are at least 100 times more active than extracts prepared from induced .lambda. lysogens in both promotion of .lambda. DNA packaging and cosmid cleaving. The ability to produce highly concentrated \*\*\*terminase\*\*\* would enormously facilitate the study of its structure and mechanism of action. These extracts are also extremely useful in techniques such as .lambda. DNA packaging, cosmid mapping and cosmid linearization to improve efficiency of integration into mouse eggs.

L4 ANSWER 10 OF 10 MEDLINE  
ACCESSION NUMBER: 82174305 MEDLINE  
DOCUMENT NUMBER: 82174305  
TITLE: Gene shuttling: moving of cloned DNA into and out of eukaryotic cells.  
AUTHOR: Lindenmaier W; Hauser H; de Wilke I G; Schutz G  
SOURCE: NUCLEIC ACIDS RESEARCH, (1982 Feb 25) 10 (4) 1243-56.  
Journal code: O8L. ISSN: 0301-5610.  
PUB. COUNTRY: ENGLAND: United Kingdom  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198208

DUPLICATE 4

AB    Successful shuttling of cloned DNA in eukaryotic cells should allow isolation of expressed genes. We tested the utility of cosmids for moving DNA into and out of eukaryotic cells. The unique cleavage of DNA at the cos site by the \*\*\*terminase\*\*\* function of lambda was exploited to maintain the linkage between the \*\*\*vector\*\*\* and inserted gene sequences, a prerequisite for successful rescue of the  
      \*\*\*transforming\*\*\* DNA from high molecular weight DNA of the eukaryotic  
      \*\*\*transformant\*\*\* . A cosmid recombinant containing the HSV thymidine kinase gene and a lambda recombinant containing the chicken thymidine kinase gene were used to test the feasibility of this method. It was found that these recombinants can be rescued with high efficiency from DNA of HAT-resistant cells.

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USPT	vector near4 circular\$7	1047	<u>L6</u>
USPT	l4 and blunt	22	<u>L5</u>
USPT	l3 and ligase	30	<u>L4</u>
USPT	l2 and phosphate	50	<u>L3</u>
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Entry 1 of 35

File: USPT

Feb 1, 2000

US-PAT-NO: 6020156

DOCUMENT-IDENTIFIER: US 6020156 A

TITLE: Method and system for biosynthesizing a desired biologically useful macromolecule in a chlorella cell and for controlling biosynthesis thereof

DATE-ISSUED: February 1, 2000

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cannons; Andrew Clive	Tampa	FL	N/A	N/A
Dawson; Hana Nenicka	Durham	NC	N/A	N/A
Solomonson; Larry Paul	Tampa	FL	N/A	N/A

US-CL-CURRENT: 435/69\_1; 435/257\_3, 435/320\_1, 435/471, 536/24\_1

## ABSTRACT:

Biosynthesis of a desired macromolecule includes inserting a promoter that encodes for an endogenously produced inducible macromolecule in a Chlorella cell into a DNA vector to form a first recombinant DNA vector. A cDNA sequence that encodes for the desired macromolecule is inserted into the first recombinant DNA vector to form a second recombinant DNA vector, which is then incorporated into the microalgae cell genome to form a transformed microalgae cell. Transcription and translation of the cDNA sequence are induced in the transformed microalgae cell to biosynthesize the desired macromolecule. Control of the method is achieved with the use of a mechanism effective in repressing an expression of the endogenously produced inducible macromolecule. Then, when desired, expression of the cDNA sequence may be induced by removing the repressing agent, thereby inducing a biosynthesis of the desired macromolecule.

30 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

<a href="#">Full</a>	<a href="#">Title</a>	<a href="#">Citation</a>	<a href="#">Front</a>	<a href="#">Review</a>	<a href="#">Classification</a>	<a href="#">Date</a>	<a href="#">Reference</a>	<a href="#">Claims</a>	<a href="#">KWC</a>	<a href="#">Image</a>
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☐ 2. Document ID: US 6015677 A

Entry 2 of 35

File: USPT

Jan 18, 2000

US-PAT-NO: 6015677  
DOCUMENT-IDENTIFIER: US 6015677 A

TITLE: Assay methods using DNA encoding mammalian phosphodiesterases

DATE-ISSUED: January 18, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Beavo; Joseph A.	Seattle	WA	N/A	N/A
Bentley; Kelley J.	Seattle	WA	N/A	N/A
Charbonneau; Harry	W. Lafayette	IN	N/A	N/A
Sonnenburg; William K.	Mountlake Terrace	WA	N/A	N/A

US-CL-CURRENT: 435/6; 435/196, 435/252.3, 435/254.21, 435/29

ABSTRACT:

The present invention relates to novel purified and isolated nucleotide sequences encoding mammalian Ca<sup>sup.2+</sup>/calmodulin stimulated phosphodiesterases (CaM-PDEs) and cyclic-GMP-stimulated phosphodiesterases (cGS-PDEs). Also provided are the corresponding recombinant expression products of said nucleotide sequences, immunological reagents specifically reactive therewith, and procedures for identifying compounds which modulate the enzymatic activity of such expression products.

4 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 3. Document ID: US 5977305 A

Entry 3 of 35

File: USPT

Nov 2, 1999

US-PAT-NO: 5977305

DOCUMENT-IDENTIFIER: US 5977305 A

TITLE: Cloning by complementation and related processes

DATE-ISSUED: November 2, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wigler; Michael H.	Lloyd Harbor	NY	N/A	N/A
Colicelli; John J.	Los Angeles	CA	N/A	N/A

US-CL-CURRENT: 530/350; 530/300

ABSTRACT:

Disclosed are methods for detecting mammalian genes encoding proteins which can function in microorganisms, particularly yeast, to modify, complement, or suppress a genetic defect associated with an identifiable phenotypic alteration or characteristic in the microorganism. Disclosed also are mammalian DNA sequences cloned by the above method, as well as polypeptide products of the expression of the DNA sequences in procaryotic or eucaryotic host cells and antibody substances which are specifically immunoreactive with said expression products. More specifically, the present invention relates to methods for cloning mammalian genes which encode products which modify, complement or suppress a genetic defect in a biochemical pathway in which cAMP participates or in a biochemical pathway which is controlled, directly or indirectly, by a RAS-related protein, to products (RNA, proteins) encoded by the mammalian genes cloned in this manner, and to antibodies which can bind the encoded proteins.

4 Claims, 16 Drawing figures

Exemplary Claim Number: 1,2,3,4

Number of Drawing Sheets: 34

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 4. Document ID: US 5976849 A

Entry 4 of 35

File: USPT

Nov 2, 1999

US-PAT-NO: 5976849

DOCUMENT-IDENTIFIER: US 5976849 A

TITLE: Human E3 ubiquitin protein ligase

DATE-ISSUED: November 2, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hustad; Carolyn Marziasz	Wilmington	DE	N/A	N/A
Ghildyal; Namit	Kennett Square	PA	N/A	N/A

US-CL-CURRENT: 435/183; 435/243, 435/254.2, 435/320.1, 435/325, 435/410, 435/455, 536/23.1, 536/23.2, 536/24.3, 536/24.31, 536/24.33

ABSTRACT:

A novel human E3 ubiquitin protein ligase is provided as well as a nucleic acid structural region which encodes the polypeptide and the amino acid residue sequence of the human biomolecule. Methods are provided to identify compounds that modulate the biological activity of the molecule and hence regulate cellular and tissue physiology.

7 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 15

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KMMC	Image
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☐ 5. Document ID: US 5976567 A

Entry 5 of 35

File: USPT

Nov 2, 1999

US-PAT-NO: 5976567  
DOCUMENT-IDENTIFIER: US 5976567 A

TITLE: Lipid-nucleic acid particles prepared via a hydrophobic lipid-nucleic acid complex intermediate and use for gene transfer

DATE-ISSUED: November 2, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wheeler; Jeffery J.	Richmond	N/A	N/A	CAX
Bally; Marcel B.	Bowen Island	N/A	N/A	CAX
Zhang; Yuan-Peng	Vancouver	N/A	N/A	CAX
Reimer; Dorothy L.	Vancouver	N/A	N/A	CAX
Hope; Michael	Vancouver	N/A	N/A	CAX
Cullis; Pieter R.	Vancouver	N/A	N/A	CAX
Scherrer; Peter	Vancouver	N/A	N/A	CAX

US-CL-CURRENT: 424/450; 435/458, 514/44

ABSTRACT:

Novel lipid-nucleic acid particulate complexes which are useful for in vitro or in vivo gene transfer are described. The particles can be formed using either detergent dialysis methods or methods which utilize organic solvents. Upon removal of a solubilizing component (i.e., detergent or an organic solvent) the lipid-nucleic acid complexes form particles wherein the nucleic acid is serum-stable and is protected from degradation. The particles thus formed have access to extravascular sites and target cell populations and are suitable for the therapeutic delivery of nucleic acids.

29 Claims, 68 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 35

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWC	Image
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☐ 6. Document ID: US 5965396 A

Entry 6 of 35

File: USPT

Oct 12, 1999

US-PAT-NO: 5965396

DOCUMENT-IDENTIFIER: US 5965396 A

TITLE: Human lymph node derived GTPase

DATE-ISSUED: October 12, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Pan; Julie Yan	Newark	DE	N/A	N/A
Egerton; Mark	Alsager	N/A	N/A	GBX
Silberstein; David Shay	Kennett Square	PA	N/A	N/A

US-CL-CURRENT: 435/69\_1; 435/243, 435/320\_1, 435/325, 435/440, 435/455, 435/6, 536/23\_1, 536/23\_2, 536/23\_5, 536/24\_32, 536/24\_5

ABSTRACT:

A novel human GTPase polypeptide intracellular molecular switch is described. A full length cDNA which encodes the signal transduction polypeptide is disclosed as well as the interior structural region and the amino acid residue sequence of the human GTPase. Methods are provided to identify compounds that modulate the biological activity of the native signal switch biomolecule and hence regulate cellular and tissue physiology.

7 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

☐ 7. Document ID: US 5959074 A

Entry 7 of 35

File: USPT

Sep 28, 1999

US-PAT-NO: 5959074

DOCUMENT-IDENTIFIER: US 5959074 A

TITLE: Products and processes for regulation of gene recombination

DATE-ISSUED: September 28, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dreyfus; David H.	Denver	CO	N/A	N/A
Gelfand; Erwin W.	Englewood	CO	N/A	N/A

US-CL-CURRENT: 530/300; 530/324

ABSTRACT:

This invention generally relates to a novel recombinogenic motif having transposase activities that is important to the regulation and function of Herpes virus replication, V(D)J recombination, and immunoglobulin class switching. The present invention also relates to a site-specific DNA binding region for V(D)J and V(D)J-like recombination signals. Disclosed are identifying characteristics of such motifs as well as methods for identifying the motifs.

2 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

☐ 8. Document ID: US 5958727 A

Entry 8 of 35

File: USPT

Sep 28, 1999

US-PAT-NO: 5958727

DOCUMENT-IDENTIFIER: US 5958727 A

TITLE: Methods for modifying the production of a polypeptide

DATE-ISSUED: September 28, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Brody; Howard	Davis	CA	N/A	N/A
Yaver; Deborah S.	Davis	CA	N/A	N/A
Lamsa; Michael	Davis	CA	N/A	N/A
Hansen; Kim	Vaerlose	N/A	N/A	DKX

US-CL-CURRENT: 435/69.1; 435/252.3, 435/254.11, 435/254.3, 435/254.4, 435/254.6, 435/254.7, 435/254.8, 435/325, 435/440, 435/455, 435/471, 435/71.1, 435/71.2

ABSTRACT:

The present invention relates to methods for modifying the production of a polypeptide, comprising: (a) introducing a nucleic acid construct into a cell, wherein the cell comprises a DNA sequence encoding a polypeptide, under conditions in which the nucleic acid construct integrates into the genome of the cell at a locus not within the DNA sequence encoding the polypeptide to produce a mutant cell, wherein the integration of the nucleic acid construct modifies the production of the polypeptide by the mutant cell relative to the cell when the mutant cell and the cell are cultured under the same conditions; and (b) identifying the mutant cell with the modified production of the polypeptide.

53 Claims, 38 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 46

☐ 9. Document ID: US 5942422 A

Entry 9 of 35

File: USPT

Aug 24, 1999

US-PAT-NO: 5942422

DOCUMENT-IDENTIFIER: US 5942422 A

TITLE: Method for generating a directed, recombinant fusion nucleic acid

DATE-ISSUED: August 24, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rothstein; Rodney	Maplewood	NJ	N/A	N/A

US-CL-CURRENT: 435/91.1; 435/252.3, 435/320.1, 435/810, 435/91.2, 435/DIG47, 536/23.1, 536/25.3

ABSTRACT:

The present invention provides for a method for generating a directed, recombinant fusion nucleic acid molecule which includes: (A) contacting a first pair of single-stranded primers with a first strand and a second strand of a first nucleic acid molecule and a second pair of single-stranded primers with a first strand and a second strand of a second nucleic acid molecule under hybridization conditions, (B) amplifying the first nucleic acid molecule and the first pair of primers and the second nucleic acid molecule and the second pair of primers under amplification conditions, separately; (C) mixing the amplification products from step (B) and the first primer of the first pair of primers and the second primer of the second pair of primers under hybridization conditions; (D) amplifying the hybridized molecules of step (C) under amplification conditions so as to generate a directed, recombinant fusion nucleic acid molecule so as to generate a directed, recombinant fusion nucleic acid molecule.

35 Claims, 23 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 19

☐ 10. Document ID: US 5872003 A

Entry 10 of 35

File: USPT

Feb 16, 1999

US-PAT-NO: 5872003

DOCUMENT-IDENTIFIER: US 5872003 A

TITLE: DNA sequencing by mass spectrometry via exonuclease degradation

DATE-ISSUED: February 16, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Koster; Hubert	Concord	MA	N/A	N/A

US-CL-CURRENT: 435/283.1; 435/286.1, 435/287.2, 435/287.3, 435/288.7

ABSTRACT:

Methods for determining the sequence of nucleic acids by cleaving the nucleic acid unilaterally from a first end with an exonuclease activity to sequentially release individual nucleotides, identifying each of the sequentially release nucleotides by mass spectrometry, and determining the sequence of the nucleic acid from the identified nucleotides are disclosed. The method is amenable to multiplexing for simultaneously determining more than one nucleic acid sequence.

21 Claims, 15 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

☐ 11. Document ID: US 5869239 A

Entry 11 of 35

File: USPT

Feb 9, 1999

US-PAT-NO: 5869239

DOCUMENT-IDENTIFIER: US 5869239 A

TITLE: Library screening method

DATE-ISSUED: February 9, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Treco; Douglas A.	Arlington	MA	N/A	N/A
Miller; Allan M.	Medford	MA	N/A	N/A

US-CL-CURRENT: 435/6; 435/477, 435/482, 435/490, 435/91\_4, 435/91\_41

ABSTRACT:

Materials and methods for homologous-recombination screening of DNA libraries constructed in a eukaryotic host and methods for homologous-recombination chromosome walking for isolating overlapping DNA sequences for building an extended physical map of a chromosomal region.

41 Claims, 18 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

☐ 12. Document ID: US 5863794 A

Entry 12 of 35

File: USPT

Jan 26, 1999

US-PAT-NO: 5863794

DOCUMENT-IDENTIFIER: US 5863794 A

TITLE: SV40 viral vectors for targeted integration into celis

DATE-ISSUED: January 26, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Strayer; David	Newtown Square	PA	N/A	N/A

US-CL-CURRENT: 435/320\_1

ABSTRACT:

A vector capable of integrating into a selected site of a cell's genome is provided which contains a replication-deficient SV40 virus and a nucleic acid sequence flanked with integration promotion sequences.

2 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

☐ 13. Document ID: US 5851765 A

Entry 13 of 35

File: USPT

Dec 22, 1998

US-PAT-NO: 5851765  
DOCUMENT-IDENTIFIER: US 5851765 A

TITLE: DNA sequencing by mass spectrometry via exonuclease degradation

DATE-ISSUED: December 22, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Koster; Hubert	Concord	MA	N/A	N/A

US-CL-CURRENT: 435/6; 250/281, 250/282, 435/91.2, 536/22.1, 536/24.3

ABSTRACT:

Methods for determining the sequence of nucleic acids by cleaving the nucleic acid unilaterally from a first end with an exonuclease activity to sequentially release individual nucleotides, identifying each of the sequentially release nucleotides by mass spectrometry, and determining the sequence of the nucleic acid from the identified nucleotides are disclosed. The method is amenable to multiplexing for simultaneously determining more than one nucleic acid sequence.

28 Claims, 15 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 14. Document ID: US 5843656 A

Entry 14 of 35

File: USPT

Dec 1, 1998

US-PAT-NO: 5843656

DOCUMENT-IDENTIFIER: US 5843656 A

TITLE: Recombinant clone selection system

DATE-ISSUED: December 1, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bridgham; John A.	Hillsborough	CA	N/A	N/A
Brandis; John	Hercules	CA	N/A	N/A
Leong; John	San Francisco	CA	N/A	N/A
Hoeprich, Jr.; Paul D.	Danville	CA	N/A	N/A

US-CL-CURRENT: 435/6; 435/252.3, 435/320.1, 435/69.1, 435/91.4, 536/23.1

ABSTRACT:

Cloning systems useful for the isolation of recombinant nucleic acid are disclosed in which the recombination of cloning-system nucleic acid and foreign nucleic acid is linked to the expression of a moiety on the surface of a host organism, the moiety being a first member of a binding pair. When recombination occurs between the nucleic acid and the foreign nucleic acid, the moiety is expressed on the surface of the host organism. The isolation of recombinant nucleic acid is then performed by attaching a second member of the binding pair to a solid support and contacting the host organism with the support. When the first member of the binding pair is expressed on the surface of the host organism, the host organism binds to the second member of the binding pair attached to the solid support, thereby selectively isolating those organisms.

10 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 15. Document ID: US 5800987 A

Entry 15 of 35

File: USPT

Sep 1, 1998

US-PAT-NO: 5800987  
DOCUMENT-IDENTIFIER: US 5800987 A

TITLE: Assay methods using DNA encoding mammalian phosphodiesterases

DATE-ISSUED: September 1, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Beavo; Joseph A.	Seattle	WA	N/A	N/A
Bentley; J. Kelley	Seattle	WA	N/A	N/A
Charbonneau; Harry	W. Lafayette	IN	N/A	N/A
Sonnenburg; William K.	Mountlake Terrace	WA	N/A	N/A

US-CL-CURRENT: 435/6; 435/196, 435/252.3, 435/254.21, 435/29

ABSTRACT:

The present invention relates to novel purified and isolated nucleotide sequences encoding mammalian Ca<sup>sup.2+</sup> /calmodulin stimulated phosphodiesterases (CaM-PDEs) and cyclic-GMP-stimulated phosphodiesterases (cGS-PDEs). Also provided are the corresponding recombinant expression products of said nucleotide sequences, immunological reagents specifically reactive therewith, and procedures for identifying compounds which modulate the enzymatic activity of such expression products.

4 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 16. Document ID: US 5801030 A

Entry 16 of 35

File: USPT

Sep 1, 1998

US-PAT-NO: 5801030

DOCUMENT-IDENTIFIER: US 5801030 A

TITLE: Methods and vectors for site-specific recombination

DATE-ISSUED: September 1, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
McVey; Duncan L.	Derwood	MD	N/A	N/A
Kovesdi; Imre	Rockville	MD	N/A	N/A

US-CL-CURRENT: 435/456; 435/320.1, 435/462, 536/23.1, 536/23.2

ABSTRACT:

The present invention provides methods for site-specific recombination in a cell, as well as vectors which can be employed in such methods. The methods and vectors of the present invention can be used to obtain persistent gene expression in a cell and to modulate gene expression.

One preferred method according to the invention comprises contacting a cell with a vector comprising an origin of replication functional in mammalian cells located between first and second recombining sites located in parallel. Another preferred method comprises, in part, contacting a cell with a vector comprising first and second recombining sites in antiparallel orientations such that the vector is internalized by the cell. In both methods, the cell is further provided with a site-specific recombinase that effects recombination between the first and second recombining sites of the vector.

47 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 17. Document ID: US 5789177 A

Entry 17 of 35

File: USPT

Aug 4, 1998

US-PAT-NO: 5789177

DOCUMENT-IDENTIFIER: US 5789177 A

TITLE: Glycoproteins, antibodies, and diagnostic kits for detection of bovine herpesvirus type 1

DATE-ISSUED: August 4, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rijsewijk; Franciscus Antonius	Amsterdam	N/A	N/A	NLX Maria Lelystad N/A N/A NLX
van Oirschot; Johannes Theodorus	Okemos	MI	N/A	N/A
Maes; Roger Kamiel				

US-CL-CURRENT: 435/7.1; 424/204.1, 424/229.1, 435/5, 435/6, 435/7.92, 435/7.93, 435/7.94

ABSTRACT:

Deletion mutant of bovine herpesvirus type 1 which has a deletion in the glycoprotein gE-gene. The mutant may further have a deletion in the thymidine kinase gene and/or the glycoprotein gI-gene, or have an insertion of a heterologous gene. Recombinant nucleic acid which comprises the gE-gene or a part thereof. Glycoprotein gE, peptides based thereon and complexes of the glycoproteins gE and gI, and antibodies against them. Vaccines and diagnostic kits comprising any one of these materials.

32 Claims, 34 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 34

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	DOC	Image
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☐ 18. Document ID: US 5789553 A

Entry 18 of 35

File: USPT

Aug 4, 1998

US-PAT-NO: 5789553  
DOCUMENT-IDENTIFIER: US 5789553 A

TITLE: Antibodies specifically immunoreactive with human brain Ca.sup.2+ /calmodulin cyclic nucleotide phosphodiesterase

DATE-ISSUED: August 4, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Beavo; Joseph A.	Seattle	WA	N/A	N/A
Bentley; J. Kelley	Seattle	WA	N/A	N/A
Charbonneau; Harry	W. Lafayette	IN	N/A	N/A
Sonnenburg; William K.	Mountlake Terrace	WA	N/A	N/A

US-CL-CURRENT: 530/388.26; 530/387.9

ABSTRACT:

The present invention relates to novel purified and isolated nucleotide sequences encoding mammalian Ca.sup.2+ /calmodulin stimulated phosphodiesterases (CaM-PDEs) and cyclic-GMP-stimulated phosphodiesterases (cGS-PDEs). Also provided are the corresponding recombinant expression products of said nucleotide sequences, immunological reagents specifically reactive therewith, and procedures for identifying compounds which modulate the enzymatic activity of such expression products.

1 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KMC	Image
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☐ 19. Document ID: US 5776752 A

Entry 19 of 35

File: USPT

Jul 7, 1998

US-PAT-NO: 5776752

DOCUMENT-IDENTIFIER: US 5776752 A

TITLE: Isolated and purified calcium/calmodulin stimulated cyclic nucleotide phosphodiesterases

DATE-ISSUED: July 7, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Beavo; Joseph A.	Seattle	WA	N/A	N/A
Bentley; J. Kelley	Seattle	WA	N/A	N/A
Charbonneau; Harry	W. Lafayette	IN	N/A	N/A
Sonnenburg; William K.	Mountlake Terrace	WA	N/A	N/A

US-CL-CURRENT: 435/196; 530/350

ABSTRACT:

The present invention relates to novel purified and isolated nucleotide sequences encoding mammalian Ca.sup.2+ /calmodulin stimulated phosphodiesterases (CaM-PDEs) and cyclic-GMP-stimulated phosphodiesterases (cGS-PDEs). Also provided are the corresponding recombinant expression products of said nucleotide sequences, immunological reagents specifically reactive therewith, and procedures for identifying compounds which modulate the enzymatic activity of such expression products.

12 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KMC	Image
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US-PAT-NO: 5773244

DOCUMENT-IDENTIFIER: US 5773244 A

TITLE: Methods of making circular RNA

DATE-ISSUED: June 30, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ares, Jr.; Manuel	Santa Cruz	CA	N/A	N/A
Ford; Ethan E.	Harbor	NY	N/A	N/A

US-CL-CURRENT: 435/69.1; 435/252.3; 435/254.2; 435/254.21; 435/320.1; 435/91.1; 435/91.21; 435/91.3; 435/91.31; 435/91.4; 435/91.42; 536/23.1

ABSTRACT:

A prototype RNA cyclase ribozyme that allows efficient production of circular RNA. Methods for modifying the prototype to produce a wide variety of custom circular RNA are detailed. The method utilizes a new plasmid which enables production of a wide variety of imaginable RNA sequences in a covalent, circular form free from intron sequences in vitro. At a particular site in the plasmid, a sequence coding for the desired circular RNA is inserted to create a new RNA cyclase ribozyme gene. RNA transcribed from RNA cyclase ribozyme genes autocatalytically converts the desired RNA sequence it contains into circular form. RNA cyclase genes may be placed into appropriate expression vectors for synthesis of circular RNA in vivo as part of ribozyme or antisense gene regulation approaches to genetic engineering.

26 Claims, 29 Drawing figures

Exemplary Claim Number: 1,13

Number of Drawing Sheets: 14

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	RWC	Image
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☐ 21. Document ID: US 5750346 A

Entry 21 of 35

File: USPT

May 12, 1998

US-PAT-NO: 5750346

DOCUMENT-IDENTIFIER: US 5750346 A

TITLE: Host organism capture

DATE-ISSUED: May 12, 1998

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bridgham; John A.	Hillsborough	CA	N/A	N/A
Brandis; John	Hercules	CA	N/A	N/A
Leong; John	San Francisco	CA	N/A	N/A
Hoeprich, Jr.; Paul D.	Danville	CA	N/A	N/A
Sloan; Charles L.	Fremont	CA	N/A	N/A
O'Neill; Roger A.	San Carlos	CA	N/A	N/A
Andre; Charles	Foster City	CA	N/A	N/A

US-CL-CURRENT: 435/6; 435/7.1, 435/7.92, 435/91.2, 436/63, 536/23.1

## ABSTRACT:

Cloning systems useful for the isolation of recombinant nucleic acid are disclosed in which the recombination of cloning-system nucleic acid and foreign nucleic acid is linked to the expression of a moiety on the surface of a host organism, the moiety being a first member of a binding pair. When recombination occurs between the nucleic acid and the foreign nucleic acid, the moiety is expressed on the surface of the host organism. The isolation of recombinant nucleic acid is then performed by attaching a second member of the binding pair to a solid support and contacting the host organism with the support. When the first member of the binding pair is expressed on the surface of the host organism, the host organism binds to the second member of the binding pair attached to the solid support, thereby selectively isolating those organisms. Other aspects of the invention include devices for the isolation of individual cells that differentially express binding moieties on their surface. The devices may be adapted for polynucleotide sequencing and analysis of polynucleotides in the isolated cells. Additional aspects of the invention include methods of using the devices for single cell isolation and analysis.

38 Claims, 23 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 22. Document ID: US 5721118 A

Entry 22 of 35

File: USPT

Feb 24, 1998

US-PAT-NO: 5721118  
DOCUMENT-IDENTIFIER: US 1118 A

TITLE: Mammalian artificial chromosomes and methods of using same

DATE-ISSUED: February 24, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Scheffler; Immo E.	Del Mar	CA	N/A	N/A

US-CL-CURRENT: 435/69.1; 435/320.1, 435/325, 435/449, 514/44, 536/23.1, 536/23.5

ABSTRACT:

The present invention provides a mammalian artificial chromosome (MAC), comprising a centromere and a unique cloning site, said MAC containing less than 0.1% of the DNA present in a normal haploid genome of the mammalian cell from which the centromere was obtained. The invention further provides a MAC, wherein the unique cloning site is a nucleic acid sequence encoding a selectable marker. The invention also provides methods of preparing a MAC. In addition, the invention provides methods of stably expressing a selectable marker in a cell, comprising introducing a MAC containing the selectable marker into the cell. The invention also provides a cell containing a MAC expressing an exogenous nucleic acid sequence and a transgenic mammal expressing a selectable marker.

17 Claims, 6 Drawing figures  
Exemplary Claim Number: 2,7,17  
Number of Drawing Sheets: 3.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 23. Document ID: US 5693511 A

Entry 23 of 35

File: USPT

Dec 2, 1997

US-PAT-NO: 5693511

DOCUMENT-IDENTIFIER: US 5693511 A

TITLE: Immortalized human fetal osteoblastic cells

DATE-ISSUED: December 2, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Harris; Steven A.	Rochester	MN	N/A	N/A
Spelsberg; Thomas C.	Rochester	MN	N/A	N/A

US-CL-CURRENT: 435/465; 435/320.1, 435/366

ABSTRACT:

Immortalized normal human fetal osteoblastic cells that express a temperature sensitive mutant of simian virus 40 large T antigen are provided.

25 Claims, 25 Drawing figures  
Exemplary Claim Number: 1  
Number of Drawing Sheets: 25

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 24. Document ID: US 5681701 A

Entry 24 of 35

File: USPT

Oct 28, 1997

US-PAT-NO: 5681701  
DOCUMENT-IDENTIFIER: US 5681701 A

TITLE: Immortalized human fetal osteoblastic cells

DATE-ISSUED: October 28, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Harris; Steven A.	Rochester	MN	N/A	N/A
Spelsberg; Thomas C.	Rochester	MN	N/A	N/A

US-CL-CURRENT: 435/6; 435/29, 435/461

ABSTRACT:

Immortalized normal human fetal osteoblastic cells that express a temperature sensitive mutant of simian virus 40 large T antigen are provided.

19 Claims, 26 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 25

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWC	Image
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☐ 25. Document ID: US 5676951 A

Entry 25 of 35

File: USPT

Oct 14, 1997

US-PAT-NO: 5676951

DOCUMENT-IDENTIFIER: US 5676951 A

TITLE: Bovine herpesvirus type 1 deletion mutants and vaccines

DATE-ISSUED: October 14, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rijsewijk; Franciscus Antonius	Amsterdam	N/A	N/A	NLX Maria Lelystad N/A N/A NLX
van Oirschot; Johannes Theodorus	Okemos	MI	N/A	N/A
Maes; Roger Kamiel				

US-CL-CURRENT: 424/229.1; 435/235.1, 435/237, 435/69.3

ABSTRACT:

A deletion mutant of bovine herpesvirus type 1 which has a deletion in the glycoprotein gE-gene and which may further have a deletion in the thymidine kinase gene and/or the glycoprotein gI-gene, or have an insertion of a heterologous gene is disclosed. Recombinant nucleic acids which encode the gE-gene or a part thereof are also disclosed, in addition to vaccines and a method of treatment.

9 Claims, 34 Drawing figures

Exemplary Claim Number: 1,9

Number of Drawing Sheets: 34

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWC	Image
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☐ 26. Document ID: US 5661006 A

Entry 26 of 35

File: USPT

Aug 26, 1997

US-PAT-NO: 5661006  
DOCUMENT-IDENTIFIER: US 1006 A

TITLE: DNA encoding the Canine coronavirus spike protein

DATE-ISSUED: August 26, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Brown; Thomas David Kay	Needingworth	N/A	N/A	GB2
Horsburgh; Brian Colin	Cambridge	N/A	N/A	GB2

US-CL-CURRENT: 435/69.3; 435/252.3, 435/320.1, 536/23.72

ABSTRACT:

The invention is related to a nucleic acid sequence & coding a Canine coronavirus (CCV) spike protein. Such a protein can be used for the immunization of dogs against CCV infection. The nucleic acid sequence encoding the CCV spike protein can be applied for the preparation of the spike protein by means of genetic engineering techniques or can be applied for the preparation of vector vaccines.

9 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWC	Image
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☐ 27. Document ID: US 5622824 A

Entry 27 of 35

File: USPT

Apr 22, 1997

US-PAT-NO: 5622824

DOCUMENT-IDENTIFIER: US 5622824 A

TITLE: DNA sequencing by mass spectrometry via exonuclease degradation

DATE-ISSUED: April 22, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
K oster; Hubert	Concord	MA	N/A	N/A

US-CL-CURRENT: 435/6; 250/282, 250/288, 422/68.1

ABSTRACT:

Methods for determining the sequence of nucleic acids by cleaving the nucleic acid unilaterally from a first end with an exonuclease activity to sequentially release individual nucleotides, identifying each of the sequentially release nucleotides by mass spectrometry, and determining the sequence of the nucleic acid from the identified nucleotides are disclosed. The method is amenable to multiplexing for simultaneously determining more than one nucleic acid sequence.

28 Claims, 15 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWC	Image
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☐ 28. Document ID: US 5602019 A

Entry 28 of 35

File: USPT

Feb 11, 1997

US-PAT-NO: 5602019  
DOCUMENT-IDENTIFIER: US 2019 A

TITLE: DNA encoding mammalian phosphodiesterases

DATE-ISSUED: February 11, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Beavo; Joseph A.	Seattle	WA	N/A	N/A
Bentley; Kelley J.	Seattle	WA	N/A	N/A
Charbonneau; Harry	W. Lafayette	IN	N/A	N/A
Sonnenburg; William K.	Seattle	WA	N/A	N/A

US-CL-CURRENT: 435/196; 435/199, 435/252.3, 435/254.2, 435/320.1, 435/325, 536/23.2

ABSTRACT:

The present invention relates to novel purified and isolated nucleotide sequences encoding mammalian Ca<sup>sup.2+</sup> /calmodulin stimulated phosphodiesterases (CaM-PDEs) and cyclic-GMP-stimulated phosphodiesterases (cGS-PDEs). Also provided are the corresponding recombinant expression products of said nucleotide sequences, immunological reagents specifically reactive therewith, and procedures for identifying compounds which modulate the enzymatic activity of such expression products.

25 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 29. Document ID: US 5595873 A

Entry 29 of 35

File: USPT

Jan 21, 1997

US-PAT-NO: 5595873

DOCUMENT-IDENTIFIER: US 5595873 A

TITLE: T. thermophila group I introns that cleave amide bonds

DATE-ISSUED: January 21, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Joyce; Gerald F.	Encinitas	CA	N/A	N/A

US-CL-CURRENT: 435/6; 435/477, 435/91.31, 536/23.1, 536/23.2

ABSTRACT:

The present invention relates to nucleic acid enzymes or enzymatic RNA molecules that are capable of cleaving a variety of bonds, including phosphodiester bonds and amide bonds, in a variety of substrates. Thus, the disclosed enzymatic RNA molecules are capable of functioning as nucleases and/or peptidases. The present invention also relates to compositions containing the disclosed enzymatic RNA molecule and to methods of making, selecting, and using such enzymes and compositions.

20 Claims, 11 Drawing figures

Exemplary Claim Number: 1,20

Number of Drawing Sheets: 7

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 30. Document ID: US 5580967 A

Entry 30 of 35

File: USPT

Dec 3, 1996

US-PAT-NO: 5580967  
DOCUMENT-IDENTIFIER: US 5580967 A

TITLE: Optimized catalytic DNA-cleaving ribozymes

DATE-ISSUED: December 3, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Joyce; Gerald F.	Encinitas	CA	N/A	N/A

US-CL-CURRENT: 536/23.2; 435/6, 435/91.31, 536/23.1, 536/24.5

ABSTRACT:

The present invention discloses nucleic acid enzymes capable of cleaving nucleic acid molecules, including single-stranded DNA, in a site-specific manner under physiologic conditions, as well as compositions including same. The present invention also discloses methods of making and using the disclosed enzymes and compositions.

8 Claims, 15 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 31. Document ID: US 5580771 A

Entry 31 of 35

File: USPT

Dec 3, 1996

US-PAT-NO: 5580771

DOCUMENT-IDENTIFIER: US 5580771 A

TITLE: DNA encoding mammalian phosphodiesterases

DATE-ISSUED: December 3, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Beavo; Joseph A.	Seattle	WA	N/A	N/A
Charbonneau; Harry	W. Lafayette	IN	N/A	N/A
Sonnenburg; William K.	Mountlake Terrace	WA	N/A	N/A

US-CL-CURRENT: 435/199; 435/196, 435/254.2, 435/320.1, 435/325, 435/365, 536/23.2

ABSTRACT:

The present invention relates to novel purified and isolated nucleotide sequences encoding mammalian Ca<sup>2+</sup>/calmodulin stimulated phosphodiesterases (CaM-PDEs) and cyclic-GMP-stimulated phosphodiesterases (cGS-PDEs). Also provided are the corresponding recombinant expression products of said nucleotide sequences, immunological reagents specifically reactive therewith, and procedures for identifying compounds which modulate the enzymatic activity of such expression products.

9 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 32. Document ID: US 5580759 A

Entry 32 of 35

File: USPT

Dec 3, 1996

US-PAT-NO: 5580759  
DOCUMENT-IDENTIFIER: US 5580759 A

TITLE: Construction of recombinant DNA by exonuclease recession

DATE-ISSUED: December 3, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Yang; Yih-Sheng	Garland	TX	N/A	N/A
Tucker; Philip W.	Dallas	TX	N/A	N/A
Capra; J. Donald	Dallas	TX	N/A	N/A

US-CL-CURRENT: 435/91.1; 435/488, 435/6, 435/91.2, 435/91.4, 435/91.5, 435/91.53

ABSTRACT:

An exonuclease-based method for joining and/or constructing two or more DNA molecules. DNA fragments containing ends complementary to those of a vector or another independent molecule were generated by the polymerase chain reaction. The 3' ends of these molecules as well as the vector DNA were then recessed by exonuclease activity and annealed in an orientation-determined manner via their complementary single-stranded regions. This recombinant DNA may be transformed directly into bacteria without a further ligase-dependent reaction. Using this approach, recombinant DNA molecules are constructed rapidly, efficiently and directionally. This method can effectively replace conventional protocols for PCR cloning, PCR SOEing, DNA subcloning and site-directed mutagenesis.

56 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	RWC	Image
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☐ 33. Document ID: US 5527896 A

Entry 33 of 35

File: USPT

Jun 18, 1996

US-PAT-NO: 5527896

DOCUMENT-IDENTIFIER: US 5527896 A

TITLE: Cloning by complementation and related processes

DATE-ISSUED: June 18, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wigler; Michael H.	Lloyd Harbor	NY	N/A	N/A
Colicelli; John J.	Los Angeles	CA	N/A	N/A

US-CL-CURRENT: 536/23.5; 435/6

ABSTRACT:

Disclosed are methods for detecting mammalian genes encoding proteins which can function in microorganisms, particularly yeast, to modify, complement, or suppress a genetic defect associated with an identifiable phenotypic alteration or characteristic in the microorganism. Disclosed also are mammalian DNA sequences cloned by the above method, as well as polypeptide products of the expression of the DNA sequences in procaryotic or eucaryotic host cells and antibody substances which are specifically immunoreactive with said expression products. More specifically, the present invention relates to methods for cloning mammalian genes which encode products which modify, complement or suppress a genetic defect in a biochemical pathway in which cAMP participates or in a biochemical pathway which is controlled, directly or indirectly, by a RAS-related protein, to products (RNA, proteins) encoded by the mammalian genes cloned in this manner, and to antibodies which can bind the encoded proteins.

6 Claims, 26 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 26

☐ 34. Document ID: US 5389527 A

Entry 34 of 35

File: USPT

Feb 14, 1995

US-PAT-NO: 5389527

DOCUMENT-IDENTIFIER: US 5389527 A

TITLE: DNA encoding mammalian phosphodiesterases

DATE-ISSUED: February 14, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Beavo; Joseph A.	Seattle	WA	N/A	N/A
Charbonneau; Harry	W. Lafayette	WA	N/A	N/A
Sonnenburg; William K.	Mountlake Terrace	WA	N/A	N/A

US-CL-CURRENT: 435/69.1; 435/196, 435/199, 435/252.3, 435/254.11, 435/320.1, 536/23.2

ABSTRACT:

The present invention relates to novel purified and isolated nucleotide sequences encoding mammalian Ca<sup>sup.2+</sup> /calmodulin stimulated phosphodiesterases (CaM-PDEs) and cyclic-GMP-stimulated phosphodiesterases (cGS-PDEs). Also provided are the corresponding recombinant expression products of said nucleotide sequences, immunological reagents specifically reactive therewith, and procedures for identifying compounds which modulate the enzymatic activity of such expression products.

13 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

☐ 35. Document ID: US 5162215 A

Entry 35 of 35

File: USPT

Nov 10, 1992

TITLE: Method of gene transfer into chickens and other avian species

DATE-ISSUED: November 10, 1992

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bosselman; Robert A.	Thousand Oaks	CA	N/A	N/A
Hu; Shaw-Fen S.	Newbury Park	CA	N/A	N/A
Nicolson; Margery A.	Pacific Palisades	CA	N/A	N/A

US-CL-CURRENT: 800/23; 435/320.1, 435/948

ABSTRACT:

A method for introducing a replication-defective retroviral vector into pluripotent stem cells of embryos of an avian species, including chickens, turkeys, quails or ducks. The method is useful for transferring nucleic acid sequences into embryonic avian cells which may differentiate into somatic or germ cells. Transfer into germ cells has been achieved to produce transgenic animals. The replication-defective retroviral vector used for transfer may be a recombinant retroviral vector containing both a retroviral derived nucleic acid sequence and a non-retroviral derived nucleic acid sequence. Examples of non-retroviral nucleic acid sequences are a neomycin resistance gene from the bacterial transposon Tn5, a herpes simplex virus thymidine kinase gene and a chicken growth hormone gene, however, any prokaryotic or eukaryotic nucleic acid sequence of interest may be used. Transgenic chickens have been produced whose cells contain and express a replication-defective retroviral vector nucleic acid sequence.

12 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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Entry 1 of 22

File: USPT

Feb 15, 2000

US-PAT-NO: 6025156

DOCUMENT-IDENTIFIER: US 6025156 A

TITLE: Topoisomerase III

DATE-ISSUED: February 15, 2000

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gwynn; Michael N.	Chester Springs	PA	N/A	N/A
Kallendar; Howard	King of Prussia	PA	N/A	N/A
Palmer; Leslie M.	Malvern	PA	N/A	N/A

US-CL-CURRENT: 435/69.1, 435/183, 435/233, 435/252.3, 435/320.1, 435/471, 530/350, 536/23.2, 536/23.7, 536/24.32

## ABSTRACT:

Topoisomerase III polypeptides and DNA and RNA encoding such Topoisomerase III polypeptides and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such Topoisomerase III for the treatment of infection, particularly bacterial infections. Antagonists against such Topoisomerase III and their use as a therapeutic to treat infections, particularly bacterial infections are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to the presence of Topoisomerase III nucleic acid sequences and the polypeptides in a host. Also disclosed are diagnostic assays for detecting polynucleotides encoding Staphylococcal Topoisomerase III and for detecting the polypeptide in a host.

25 Claims, 2 Drawing figures

Exemplary Claim Number: 20,22,23

Number of Drawing Sheets: 3

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 2. Document ID: US 6013505 A

Entry 2 of 22

File: USPT

Jan 11, 2000

US-PAT-NO: 6013505  
DOCUMENT-IDENTIFIER: US 6013505 A

TITLE: Topoisomerase I

DATE-ISSUED: January 11, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gwynn; Michael N.	Chester Springs	PA	N/A	N/A
Kallendar; Howard	King of Prussia	PA	N/A	N/A
Palmer; Leslie M.	Malvern	PA	N/A	N/A

US-CL-CURRENT: 435/233; 435/252\_3, 435/320\_1, 536/23\_2

ABSTRACT:

Topoisomerase I polypeptides and DNA and RNA encoding such Topoisomerase I polypeptides and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such Topoisomerase I for the treatment of infection, particularly bacterial infections. Antagonists against such Topoisomerase I and their use as a therapeutic to treat infections, particularly bacterial infections are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to the presence of Topoisomerase I nucleic acid sequences and the polypeptides in a host. Also disclosed are diagnostic assays for detecting polynucleotides encoding Staphylococcal Topoisomerase I and for detecting the polypeptide in a host.

25 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 3. Document ID: US 6007989 A

Entry 3 of 22

File: USPT

Dec 28, 1999

US-PAT-NO: 6007989

DOCUMENT-IDENTIFIER: US 6007989 A

TITLE: Methods of screening for compounds that derepress or increase telomerase activity

DATE-ISSUED: December 28, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
West; Michael D.	San Carlos	CA	N/A	N/A
Harley; Calvin B.	Palo Alto	CA	N/A	N/A
Weinrich; Scott L.	San Francisco	CA	N/A	N/A
Strahl; Catherine M.	San Francisco	CA	N/A	N/A
McEachern; Michael J.	San Francisco	CA	N/A	N/A
Shay; Jerry	Dallas	TX	N/A	N/A
Wright; Woodring E.	Arlington	TX	N/A	N/A
Blackburn; Elizabeth H.	San Francisco	CA	N/A	N/A
Kim; Nam Woo	Sunnyvale	CA	N/A	N/A
Vaziri; Homayoun	Toronto	N/A	N/A	CAX

US-CL-CURRENT: 435/6; 435/15, 435/375, 435/4, 435/7.2, 435/91.1, 435/91.2

ABSTRACT:

Method and compositions are provided for the determination of telomere length and telomerase activity, as well as the ability to increase or decrease telomerase activity in the treatment of proliferative diseases. Particularly, primers are elongated under conditions which minimize interference from other genomic sequences, so as to obtain accurate determinations of telomeric length or telomerase activity. In addition, compositions are provided for intracellular inhibition of telomerase activity and means are shown for slowing or reversing the loss of telomeric repeats in aging cells.

28 Claims, 43 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 43

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWC	Image
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☐ 4. Document ID: US 6001645 A

Entry 4 of 22

File: USPT

Dec 14, 1999

US-PAT-NO: 6001645  
DOCUMENT-IDENTIFIER: US 6001645 A

TITLE: Thermophilic DNA polymerases from thermotoga neapolitana

DATE-ISSUED: December 14, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Slater; Michael R.	Madison	WI	N/A	N/A
Huang; Fen	Madison	WI	N/A	N/A
Hartnett; James R.	Fitchburg	WI	N/A	N/A

US-CL-CURRENT: 435/320.1; 435/194, 536/23.2

ABSTRACT:

The present invention relates to thermostable DNA polymerases derived from the hyperthermophilic eubacteria, and Thermotoga neapolitana in particular. The present invention provides means for isolating and producing the enzymes from these thermostable DNA polymerases, which are useful in many recombinant DNA techniques, especially such techniques as thermal cycle sequencing and nucleic acid amplification.

3 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 5. Document ID: US 5981225 A

Entry 5 of 22

File: USPT

Nov 9, 1999

US-PAT-NO: 5981225

DOCUMENT-IDENTIFIER: US 5981225 A

TITLE: Gene transfer vector, recombinant adenovirus particles containing the same, method for producing the same and method of use of the same

DATE-ISSUED: November 9, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kochanek; Stefan	Cologne	N/A	N/A	DEX
Schiedner; Gudrun	Cologne	N/A	N/A	DEX

US-CL-CURRENT: 435/69.1; 435/320.1, 435/456, 435/457, 536/23.5, 536/23.72, 536/24.1

ABSTRACT:

A gene transfer vector comprising adenovirus inverted terminal repeats, at least one adenovirus packaging signal, and an adenoviral VAI gene and/or VAI gene; recombinant adenovirus particles containing the same; a method for producing the same and a method of use of the same to introduce and express a foreign gene in adenovirus target cells, is disclosed.

36 Claims, 4 Drawing figures

Exemplary Claim Number: 29

Number of Drawing Sheets: 4

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 6. Document ID: US 5962303 A

Entry 6 of 22

File: USPT

Oct 5, 1999

US-PAT-NO: 5962303  
DOCUMENT-IDENTIFIER: US 2303 A

TITLE: Topoisomerase III

DATE-ISSUED: October 5, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gwynn; Michael N.	Chester Springs	PA	N/A	N/A
Kallendar; Howard	King of Prussia	PA	N/A	N/A

US-CL-CURRENT: 435/233; 435/252.3, 435/254.11, 435/320.1, 435/325

ABSTRACT:

Topoisomerase III polypeptides and DNA and RNA encoding such Topoisomerase III polypeptides and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such Topoisomerase III for the treatment of infection, particularly bacterial infections. Antagonists against such Topoisomerase III and their use as a therapeutic to treat infections, particularly bacterial infections are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to the presence of Topoisomerase III nucleic acid sequences and the polypeptides in a host. Also disclosed are diagnostic assays for detecting polynucleotides encoding Streptococcal Topoisomerase III and for detecting the polypeptide in a host.

29 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 7. Document ID: US 5916752 A

Entry 7 of 22

File: USPT

Jun 29, 1999

US-PAT-NO: 5916752

DOCUMENT-IDENTIFIER: US 5916752 A

TITLE: Telomerase screening methods

DATE-ISSUED: June 29, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gottschling; Daniel E.	Chicago	IL	N/A	N/A
Singer; Miriam S.	Chicago	IL	N/A	N/A

US-CL-CURRENT: 435/6; 435/5, 435/69.1, 435/91.1, 435/91.2, 436/501

ABSTRACT:

Disclosed are various methods, compositions and screening assays connected with telomerase, including genes encoding the template RNA of *S. cerevisiae* telomerase and various telomerase-associated polypeptides.

56 Claims, 15 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 15

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 8. Document ID: US 5910414 A

Entry 8 of 22

File: USPT

Jun 8, 1999

US-PAT-NO: 5910414  
DOCUMENT-IDENTIFIER: US 0414 A

TITLE: Topoisomerase I of streptococcus pneumoniae

DATE-ISSUED: June 8, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gwynn; Michael N.	Chester Springs	PA	N/A	N/A
Kallender; Howard	King of Prussia	PA	N/A	N/A

US-CL-CURRENT: 435/6; 435/252.3, 435/254.11, 435/320.1, 435/325, 435/69.1, 536/23.7, 536/24.32

ABSTRACT:

topoisomerase I polypeptides and DNA and RNA encoding such topoisomerase I polypeptides and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such topoisomerase I for the treatment of infection, particularly bacterial infections. Antagonists against such topoisomerase I and their use as a therapeutic to treat infections, particularly bacterial infections are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to the presence of topoisomerase I nucleic acid sequences and the polypeptides in a host. Also disclosed are diagnostic assays for detecting polynucleotides encoding streptococcal topoisomerase I and for detecting the polypeptide in a host.

11 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	RWMC	Image
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☐ 9. Document ID: US 5856090 A

Entry 9 of 22

File: USPT

Jan 5, 1999

US-PAT-NO: 5856090

DOCUMENT-IDENTIFIER: US 5856090 A

TITLE: DNA-methylase linking reaction

DATE-ISSUED: January 5, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Epstein; David M.	San Diego	CA	N/A	N/A

US-CL-CURRENT: 435/6; 435/320.1, 435/69.7, 435/7.1, 435/91.2, 435/91.4, 435/91.5, 536/23.1

ABSTRACT:

The activity of sequence-specific DNA binder proteins, such as DNA methylases, provides a method of obtaining a covalent linkage between a nucleic acid segment and a polypeptide determinant encoded by the nucleic acid segment. The polypeptide determinant is expressed as a fusion protein together with the DNA methylase, which binds in vivo to a cytidine suicide analog when present in a nucleotide sequence. A plasmid suitable for use in this linkage reaction can comprise: (1) a gene fusion construct including a gene encoding a DNA methylase and a gene encoding a polypeptide determinant; (2) a promoter for transcription of the gene fusion construct as messenger RNA; and (3) a methylase conjugation element linked to the gene fusion sequence, the methylase conjugation element including a methylase binding site having at least one copy of a nucleotide sequence including a cytidine suicide analog capable of irreversibly binding the DNA methylase. The plasmid can form a plasmid-polypeptide determinant conjugate. The plasmids and methods of the present invention are useful for in vitro evolution of proteins.

51 Claims, 38 Drawing figures

Exemplary Claim Number: 36

Number of Drawing Sheets: 33

☐ 10. Document ID: US 5843659 A

Entry 10 of 22

File: USPT

Dec 1, 1998

US-PAT-NO: 5843659

DOCUMENT-IDENTIFIER: US 5843659 A

TITLE: Apoptosis gene EI24, compositions, and methods of use

DATE-ISSUED: December 1, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lehar; Sophie M.	Berlin	MA	N/A	N/A
Guild; Braydon C.	Concord	MA	N/A	N/A

US-CL-CURRENT: 435/6; 435/320.1, 435/325, 435/69.1, 435/91.4, 536/23.5

ABSTRACT:

Disclosed is the isolation and characterization of EI24, a novel gene whose 2.4 kb mRNA is induced following etoposide treatment. Induction of EI24 mRNA by etoposide required expression of wild-type p53. Overexpression of functional p53 was sufficient to induce expression of the EI24 mRNA. The EI24 mRNA was also induced in a p53-dependent manner by ionizing irradiation of primary murine thymocytes. The invention is thus directed to an isolated EI24 protein, nucleotide sequences coding for and regulating expression of the protein, antibodies directed against the protein, and recombinant vectors and host cells containing the genetic sequences coding for and regulating the expression of the protein sequence. The invention is also directed to genomic DNA, cDNA, and RNA encoding the EI24 protein sequence and to corresponding antisense RNA sequences. Antibodies can be used to detect EI24 in biological specimens, including, for example, human tissue samples. The present invention is further directed to methods of treating degenerative disorders characterized in inappropriate cell proliferation or inappropriate cell death. The present invention is further directed to methods for diagnosing degenerative disorders characterized in inappropriate cell proliferation or inappropriate cell death, as well as methods for monitoring the progress of such degenerative disorders.

18 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

☐ 11. Document ID: US 5837535 A

Entry 11 of 22

File: USPT

Nov 17, 1998

US-PAT-NO: 5837535  
DOCUMENT-IDENTIFIER: US 7535 A

TITLE: Neuronal-neonatal gene: neuronatin

DATE-ISSUED: November 17, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Joseph; Rajiv	Birmingham	MI	N/A	N/A
Dou; Dexian	Dearborn	MI	N/A	N/A

US-CL-CURRENT: 435/325; 435/252.3, 435/320.1, 435/357, 435/368, 536/23.1, 536/24.1

ABSTRACT:

The present invention is an isolated and purified DNA sequence which encodes a vertebrate mRNA for a neuron specific protein, neuronatin. The mRNA is selectively expressed in brain tissue during rapid brain growth when there is a surge in neuronal proliferation and migration and is repressed in adult tissue. In the human, the genomic DNA is as set forth in SEQ ID No:6 and the cDNA has a nucleotide sequence as set forth in SEQ ID No:5, with the gene mapped to human chromosome 20q11.2-12. The deduced protein is a proteolipid that appears to have a role in ion channel regulation during brain development.

10 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWOC	Image
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☐ 12. Document ID: US 5834202 A

Entry 12 of 22

File: USPT

Nov 10, 1998

US-PAT-NO: 5834202

DOCUMENT-IDENTIFIER: US 5834202 A

TITLE: Methods for the isothermal amplification of nucleic acid molecules

DATE-ISSUED: November 10, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Auerbach; Jeffrey I.	Rockville	MD	N/A	N/A

US-CL-CURRENT: 435/6; 435/320.1, 435/91.1, 435/91.2, 536/23.1, 536/24.2, 536/24.33

ABSTRACT:

Methods for amplifying a nucleic acid molecule which employs a single primer, and in which the amplification is performed under isothermal conditions. The invention also includes kits containing reagents for conducting the method.

24 Claims, 19 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 19

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWOC	Image
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☐ 13. Document ID: US 5834234 A

Entry 13 of 22

File: USPT

Nov 10, 1998

US-PAT-NO: 5834234  
DOCUMENT-IDENTIFIER: US 5834234 A

TITLE: Apoptosis associated protein Bbk

DATE-ISSUED: November 10, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gallo; Gregory J.	Reading	MA	N/A	N/A

US-CL-CURRENT: 435/69.1; 435/243, 435/375, 435/70.1, 435/71.1, 530/324, 530/325, 530/326, 530/327, 530/350

ABSTRACT:

The present invention is directed to an isolated Bbk protein, nucleotide sequences coding for and regulating expression of the protein, antibodies directed against the protein, and recombinant vectors and host cells containing the genetic sequences coding for and regulating the expression of the protein sequence. The invention is also directed to genomic DNA, cDNA, and RNA encoding the Bbk protein sequence and to corresponding antisense RNA sequences. Antibodies can be used to detect Bbk in biological specimens, including, for example, human tissue samples. The present invention is further directed to methods of treating degenerative disorders characterized in inappropriate cell proliferation or inappropriate cell death. The present invention is further directed to methods for diagnosing degenerative disorders characterized in inappropriate cell proliferation or inappropriate cell death, as well as methods for monitoring the progress of such degenerative disorders.

5 Claims, 20 Drawing figures  
Exemplary Claim Number: 4  
Number of Drawing Sheets: 17

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KMC	Image
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☐ 14. Document ID: US 5766891 A

Entry 14 of 22

File: USPT

Jun 16, 1998

US-PAT-NO: 5766891

DOCUMENT-IDENTIFIER: US 5766891 A

TITLE: Method for molecular cloning and polynucleotide synthesis using vaccinia DNA topoisomerase

DATE-ISSUED: June 16, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Shuman; Stewart	New York	NY	N/A	N/A

US-CL-CURRENT: 435/91.41; 435/488, 435/489, 435/91.1, 435/91.4, 435/91.5

ABSTRACT:

This invention provides a modified vaccinia topoisomerase enzyme containing an affinity tag which is capable of facilitating purification of protein-DNA complexes away from unbound DNA. This invention further provides a modified sequence specific topoisomerase enzyme. This invention provides a method of ligating duplex DNAs, a method of molecular cloning of DNA, a method of synthesizing polynucleotides, and a method of gene targeting. Lastly, this invention provides a recombinant DNA molecule composed of segments of DNA which have been joined ex vivo by the use of a sequence specific topoisomerase and which has the capacity to transform a suitable host cell comprising a DNA sequence encoding polypeptide activity.

11 Claims, 21 Drawing figures  
Exemplary Claim Number: 1  
Number of Drawing Sheets: 12

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KMC	Image
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☐ 15. Document ID: US 5733733 A

Entry 15 of 22

File: USPT

Mar 31, 1998

US-PAT-NO: 5733733

DOCUMENT-IDENTIFIER: US 5733733 A

TITLE: Methods for the isothermal amplification of nucleic acid molecules

DATE-ISSUED: March 31, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Auerbach; Jeffrey I.	Rockville	MD	N/A	N/A

US-CL-CURRENT: 435/6; 435/320.1, 435/5, 435/91.1, 435/91.2, 536/23.1, 536/24.3, 536/24.33

ABSTRACT:

Methods for amplifying a nucleic acid molecule which employs a single primer, and in which the amplification is performed under isothermal conditions. The invention also includes kits containing reagents for conducting the method.

26 Claims, 19 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 19

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	Image
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☐ 16. Document ID: US 5720928 A

Entry 16 of 22

File: USPT

Feb 24, 1998

US-PAT-NO: 5720928

DOCUMENT-IDENTIFIER: US 5720928 A

TITLE: Image processing and analysis of individual nucleic acid molecules

DATE-ISSUED: February 24, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Schwartz; David C.	New York	NY	N/A	N/A

US-CL-CURRENT: 422/186; 422/129, 422/55, 422/58, 422/99, 435/6

ABSTRACT:

A method for observing and determining the size of individual molecules and for determining the weight distribution of a sample containing molecules of varying size, which involves placing a deformable or nondeformable molecule in a medium, subjecting the molecule to an external force, thereby causing conformational and/or positional changes, and then measuring these changes. Preferred ways to measure conformational and positional changes include: (1) determining the rate at which a deformable molecule returns to a relaxed state after termination of the external force, (2) determining the rate at which a molecule becomes oriented in a new direction when the direction of the perturbing force is changed, (3) determining the rate at which a molecule rotates, (4) measuring the length of a molecule, particularly when it is at least partially stretched, or (5) measuring at least one diameter of a spherical or ellipsoidal molecule. Measurements of relaxation, reorientation, and rotation rates, as well as length and diameter can be made using a light microscope connected to an image processor. Molecule relaxation, reorientation and rotation also can be determined using a microscope combined with a spectroscopic device. The invention is particularly useful for measuring polymer molecules, such as nucleic acids, and can be used to determine the size and map location of restriction digests. Breakage of large polymer molecules mounted on a microscope slide is prevented by condensing the molecules before mounting and unfolding the molecules after they have been placed in a matrix.

3 Claims, 124 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 52

☐ 17. Document ID: US 5698686 A

Entry 17 of 22

File: USPT

Dec 16, 1997

US-PAT-NO: 5698686

DOCUMENT-IDENTIFIER: US 5698686 A

TITLE: Yeast telomerase compositions

DATE-ISSUED: December 16, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gottschling; Daniel E.	Chicago	IL	N/A	N/A
Singer; Miriam S.	Chicago	IL	N/A	N/A

US-CL-CURRENT: 536/23\_1; 435/6, 435/91\_2, 536/22\_1, 536/24\_3, 536/24\_31, 536/24\_33

ABSTRACT:

Disclosed are various methods, compositions and screening assays connected with telomerase, including genes encoding the template RNA of *S. cerevisiae* telomerase and various telomerase-associated polypeptides.

71 Claims, 15 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 15

☐ 18. Document ID: US 5672686 A

Entry 18 of 22

File: USPT

Sep 30, 1997

US-PAT-NO: 5672686

DOCUMENT-IDENTIFIER: US 5672686 A

TITLE: Bcl-Y - specific antibodies

DATE-ISSUED: September 30, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chittenden; Thomas D.	Brookline	MA	N/A	N/A

US-CL-CURRENT: 530/387\_9, 530/388\_2, 530/389\_1, 530/391\_3

ABSTRACT:

The present invention is directed to an isolated Bcl-Y protein, nucleotide sequences coding for and regulating expression of the protein, antibodies directed against the protein, and recombinant vectors and host cells containing the genetic sequences coding for and regulating the expression of the protein sequence. The invention is also directed to genomic DNA, cDNA, and RNA encoding the Bcl-Y protein sequence and to corresponding antisense RNA sequences. Antibodies can be used to detect Bcl-Y in biological specimens, including, for example, human tissue samples. The present invention is further directed to methods of treating degenerative disorders characterized in inappropriate cell proliferation or inappropriate cell death. The present invention is further directed to methods for diagnosing degenerative disorders characterized in inappropriate cell proliferation or inappropriate cell death, as well as methods for monitoring the progress of such degenerative disorders.

6 Claims, 17 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 16

☐ 19. Document ID: US 5645986 A

Entry 19 of 22

File: USPT

Jul 8, 1997

US-PAT-NO: 5645986

DOCUMENT-IDENTIFIER: US 5645986 A

TITLE: Therapy and diagnosis of conditions related to telomere length and/or telomerase activity

DATE-ISSUED: July 8, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
West; Michael D.	San Carlos	CA	N/A	N/A
Harley; Calvin B.	Palo Alto	CA	N/A	N/A
Strahl; Catherine M.	San Francisco	CA	N/A	N/A
McEachern; Michael J.	San Francisco	CA	N/A	N/A
Shay; Jerry	Dallas	TX	N/A	N/A
Wright; Woodring E.	Arlington	TX	N/A	N/A
Blackburn; Elizabeth H.	San Francisco	CA	N/A	N/A
Vaziri; Homayoun	Toronto	N/A	N/A	CAX

US-CL-CURRENT: 435/6; 435/183, 435/184, 435/194, 435/91.2, 436/63, 536/24.31, 536/24.33

ABSTRACT:

Method and compositions are provided for the determination of telomere length and telomerase activity, as well as the ability to increase or decrease telomerase activity in the treatment of proliferative diseases. Particularly, primers are elongated under conditions which minimize interference from other genomic sequences, so as to obtain accurate determinations of telomeric length or telomerase activity. In addition, compositions are provided for intracellular inhibition of telomerase activity and means are shown for slowing or reversing the loss of telomeric repeats in aging cells.

27 Claims, 55 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 43

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 20. Document ID: US 5614389 A

Entry 20 of 22

File: USPT

Mar 25, 1997

US-PAT-NO: 5614389

DOCUMENT-IDENTIFIER: US 5614389 A

TITLE: Methods for the isothermal amplification of nucleic acid molecules

DATE-ISSUED: March 25, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Auerbach; Jeffrey I.	Rockville	MD	N/A	N/A

US-CL-CURRENT: 435/91.2; 435/6, 435/91.1

ABSTRACT:

Methods for amplifying a nucleic acid molecule which employs a single primer, and in which the amplification is performed under isothermal conditions. The invention also includes kits containing reagents for conducting the method.

20 Claims, 19 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 19

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 21. Document ID: US 5591609 A

Entry 21 of 22

File: USPT

Jan 7, 1997

US-PAT-NO: 5591609

DOCUMENT-IDENTIFIER: US 5591609 A

TITLE: Methods for the isothermal amplification of nucleic acid molecules

DATE-ISSUED: January 7, 1997

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Auerbach; Jeffrey I.	Rockville	MD	N/A	N/A

US-CL-CURRENT: 435/91.2; 435/6, 435/91.1, 435/91.5

## ABSTRACT:

Methods for amplifying a nucleic acid molecule which employs a single primer, and in which the amplification is performed under isothermal conditions. The invention also includes kits containing reagents for conducting the method.

15 Claims, 18 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 16

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Image
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☐ 22. Document ID: US 5354668 A

Entry 22 of 22

File: USPT

Oct 11, 1994

US-PAT-NO: 5354668

DOCUMENT-IDENTIFIER: US 5354668 A

TITLE: Methods for the isothermal amplification of nucleic acid molecules

DATE-ISSUED: October 11, 1994

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Auerbach; Jeffrey I.	Rockville	MD	20850	N/A

US-CL-CURRENT: 435/91.1; 435/6

## ABSTRACT:

Methods for amplifying a nucleic acid molecule which employs a single primer, and in which the amplification is performed under isothermal conditions. The invention also includes kits containing reagents for conducting the method.

22 Claims, 14 Drawing figures

Exemplary Claim Number: 20

Number of Drawing Sheets: 14

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